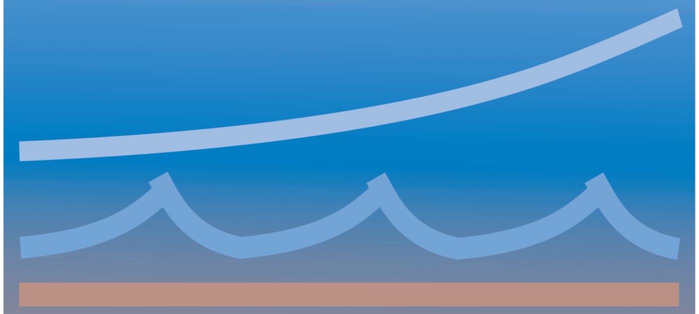

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Aim and Scope

The objective of the *Journal of Residuals Science & Technology* (JRS&T) is to provide a forum for technical research on the management and disposal of residuals from pollution control activities. The Journal publishes papers that examine the characteristics, effects, and management principles of various residuals from such sources as wastewater treatment, water treatment, air pollution control, hazardous waste treatment, solid waste, industrial waste treatment, and other pollution control activities. Papers on health and the environmental effects of residuals production, management, and disposal are also welcome.

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Editorial

Research

EDITORIAL

Hello all Journal of Residuals Science and Technology (JRS&T) Readers,

The journal would like to take the opportunity to thank very much Dr. Azize Ayol, Dr. Banu Ormeci, and Dr. Leonard Angelique. They provided the JRS&T with conference-series articles from the fourth European Conference on Sludge Management (ECSM 2014) held on May 26–27, 2014 in İzmir, Turkey (www.ecsm2014.org).

Please pardon major delay with this year's journal issues.

Since 2008, the journal has been exploring interdisciplinary papers. Topics during this time have been related to management, disposal, and research of residuals from pollution and pollution control activities including characteristics, effects, and management principles for various residuals and pollution mainly from wastewater treatment, water treatment, air pollution control, hazardous waste treatment, solid wastes handling, industrial waste treatment, and other pollution control activities. Public health and environmental effects of residuals production, management, disposal, remediation, and research have been included also. The journal's board has specialty and interdisciplinary experience in areas of sludge management, biosolids, hydrology and hydraulics, water quality and quantity, environmental sciences (water, soil, and air), environmental engineering, biology, chemistry, physics, and more. Some examples of recent topics in the journal include:

- treatment of sludge liquor;
- energy from sludge management's “Carbon Footprint;”
- energy utilization in sludge management;
- handling of residuals in the developing world versus in the developed world;
- economy of source separated waste streams in third world countries;
- biosolids’ management, treatment and disposal, and land application;
- renewable energy from biomass;
- energy self-sufficiency and lifecycle processes;
- ecosystem restoration with biosolids;
- and ways to minimize sludge/biosolids production.

Papers on controversial issues such as regrowth/reactiviation of pathogens in biosolids and new processes have been explored. The best interest of the journal and related industry is for us to continue to develop upon these topics as well as to explore new and beneficial topics.

It is the JRS&T Editorial Board’s experience guiding the journal’s direction. The boards’ experience includes the following and more:

- sludge management and remediation
  —conditioning
  —rheology or dewatering
  —watering or thickening
  —digestion
  —sludge liquor treatment
  —bioflocculation
  —anaerobic digestion
  —developing world municipal and industrial water treatment and sludge management
  —chemistry (e.g., odor, nutrient, polymer, and surfactant) and colloid and interfacial chemistry including electrokinetic phenomena
  —and more
- biosolids management, treatment and disposal, and land application;
- industrial reuse;
- drinking water, water and wastewater disinfection, anaerobic digestion, and stability;

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- identification of anthropogenic compounds and elucidation of compound fate, bioavailability, and effects in aquatic environments;
- sustainable waste, water, and energy management in "green buildings;"
- greenhouse gas emissions assessment;
- alternative energy generation and use;
- microbiology and organic micro-contaminants;
- ecological effects;
- emerging contaminants and pollutants;
- fate and persistence of contaminants;
- management of biodegradable organic wastes;
- landfill mining;
- technology and public policy issues;
- advanced UV processes;
- biomethane form animal wastes, general bioenergy systems analysis, mechanical and agricultural engineering design;
- composting;
- ambient air toxic pollutants monitoring and assessment;
- dry deposition modeling;
- fresh water systems, limnology, hydrology and hydraulics, and water quality and quantity;
- GIS, remote sensing, GPS, and mobile technologies; and
- natural resources, environmental sciences, environmental engineering, geology, earth sciences, agronomy, and more.

Board, please forgive me for experience I have not included here. These are examples meant to inspire members to promote their interests and relationships.

The journal has slightly expanded its editorial board and would like to both increase number of submissions and number of editorial board members in 2014 and 2015. Please submit ideas, opportunities, and suggestions to me at pduncan@unt.edu.

Leaving on a final note, if it is true success is not final, failure is not fatal, and courage to continue is what truly counts, then onwards and upwards we march. Please enjoy this issue.

Best regards,

Dr. P. Brent Duncan
Editor-in-Chief

Heavy Metals Transfer from Soil to Grass Types in Tannery Sludge Compost Amended Soil

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ABSTRACT: The Cr, Cu, Zn, and Ni accumulations in three different grass types were investigated with doses of 0% (control), 5%, 10%, 25%, and 50% tannery sludge compost (TSC) application to soil. The pot experiments showed that 5% and 10% TSC amendment enhanced the grass seed mixture (GSM) and Ecopalmix species while 50% TSC disrupted the plant growth. The TSC addition considerably increased the Cr concentrations in plants depending on the extreme Cr level in the TSC. The order of metals mobility according to the transfer coefficient (T_c) values was found as Zn > Cu > Cr > Ni.

INTRODUCTION

COMPOSTING has been recognized as one of the most economic and environmental friendly applications, since it provides the recovery of the organic wastes, and the final product compost is widely used in agricultural activities as soil stabilizing material. Mature compost which is adequately decomposed is a rich source of organic matter, carbon, nitrogen, phosphorus, potassium and numerous microelements. Recently, the utilization of industrial sludge composts in addition to the domestic solid waste composts is gaining popularity by means of waste disposal and soil stabilization (Schaub and Leonard, 1996; Briski *et al.*, 2003; Araijo and Monterio, 2005; Gupta and Sinha, 2006).

Composted bio-sludge carries a potential to be used as fertilizer, since it usually contains high organic matter. However, industrial sludge may have negative impacts on the environment by creating secondary pollution due to the salts and compounds existing. It is known that tannery sludge may contain heavy metals, especially Cr compounds, as a result of the tanning process. The accumulation of these heavy metals in the soil and the crops is another issue, which is related with the compost application ratio, plant tolerance and uptake, and the chemical stability of the chromium in the media.

The utilization of the tannery sludge compost (TSC) in agricultural activities has been subject to various studies in the literature. In a study conducted in Brazil, tannery sludge compost was mixed with soil in various ratios and the growth of Capsium plants was observed. In this study, the plants showed tolerance under elevated heavy metal concentrations in soil (Silva *et al.*, 2010). A similar study was completed in China to observe the effect of tannery sludge compost amended in different ratios to the soil and they grew wheat and paddy rice in pots. Their results showed that, under certain Cr concentrations, the utilization of the sludge compost did not show negative impacts on the plant growth consumability (Ju *et al.*, 2006).

TSC can be counted as an efficient soil stabilizing material due to its high organic content. However, its application on soil may pose the accumulation of the heavy metals in soil and plant tissue, and can link with the food supply chain. To avoid such case, the final compost product should be utilized for the growth of non-consumable plants such as horticulture plants or the recreational grass which do not enter the food chain of humans and animals. As it is well known, refuge sites in the traffic lines, recreational sites and golf courses are the large areas covered with mainly grass. A suitable rate of compost application to such areas may improve the soil quality as well as increasing the grass germination and development. The current study aims to examine (1) the determination of the availability of compost application for the proper grass seed germination, (2) the heavy metals accumulation in three grass types when tannery sludge compost is applied to soil in different ratios, and (3) the soil/plant transfer coefficients of Cr, Cu, Ni, and Zn

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for the plants and tannery sludge compost doses to assess the metals availability. The effects of TSC amendment on the plant growth will also be investigated and evaluated in order to suggest the optimum dose to be applied.

MATERIALS AND METHODS

Experimental Setup

The commercial leaf mould soil was taken from a florist shop in Izmir, Aegean region (Turkey). The tannery sludge compost which is used in the experiments was obtained from a leather industry zone in Marmara region (Turkey). The compost is produced of the tannery sludge coming from the treatment plant of the industrial zone. Both the soil and the TSC were passed through a 2-mm sieve. The soil was amended with TSC at rates of 0%(control), 5%, 10%, 25%, and 50% (w/w). 300 grams of soil/TSC mixtures were packed into the pots of 0.5 kg capacity. Three different grass types were used in the experiments; Grass Seed Mixture (GSM)(including 60% *Festuca arundinacca*, 10% *Lolium perenne*-topgun type, 20% *Lolium perenne*-Jackento type, 10% *Festuca rubra rubra*), Ecopalmix (15% *Lolium perenne*-Esquire type, 20% *Lolium perenne*-Sunshine type, 20% *Festuca rubra rubra*-Corail type, 20% *Festuca arundinacea*-Debussy type, 20% *Festuca arundinacea*-Eldorado type,5% poa pratensi), and Pimpernel (100% *Anagellis Arvensis*). Seeds of three grass types were sown in the pots (0.5 g seeds per pot) and the pots were kept in the laboratory under normal room conditions. Each experimental pot was arranged separately and there were three replicates for every amendment and grass type. The pots were irrigated until the saturation level at the beginning of the experimental trial period. Regular irrigations were applied every other day in order to provide the sufficient growth of the grasses. Plants were harvested on the 12nd, 21st, and finally 45th day of the experimental period and combined together at the end. The harvested plants were weighed and air dried until a constant weight was obtained and they were kept in plastic bags for the heavy metal analysis. The experimental period ended at the end of 45 days.

Analytical Methods

The pH values of the soil, TSC, and the mixture in the pots were analyzed in H_2O suspensions due to the EPA Method 9045 C (USEPA, 1995). The salinity of the soil samples were determined by using the argentometric titrations according to the Standard Methods (APHA, 1992). The gravimetric method was used for the determination of the water contents. The organic matter (OM) ratios of the mixtures in the pots were determined according to the Standard Methods (Franson *et al.*, 1992) All these analyses for each sample were duplicated and the mean values are reported.

For the extraction of the heavy metals, conventional aqua regia digestion was performed in 250 ml glass beakers covered with watch glasses. A well-mixed sample of 0.50 g was digested in 12 ml of aqua regia on a hotplate for 3 h at 1100°C. After evaporation to near dryness, the sample was diluted with 20 mL of 2% (v/v with H_2O) nitric acid and transferred into a 100 ml volumetric flask after filtering through Whatman no.42 paper and diluted to 100 ml with distilled water (Chen and Ma, 2001). The same extraction procedure was applied to the soil mixtures and the plants. The liquid extracts were kept in plastic bottles and kept at 40°C prior to the analysis. All soil and plant material digests were analyzed for Cr, Cu, Zn, and Ni concentrations by using an inductively coupled plasma-optical emission spectrometer (ICP-OES) (Perkin Elmer Inc. Optima 2100 DV, USA).

Calculation of the Transfer Coefficients (T_c)

The T_c value gives an indication of the mobility of the elements and this is particularly important for heavy metals (Antoniadis and Alloway, 2001). The T_c is given by the formula $T_c = [M]_{plant}/[M]_{soil}$, where $[M]_{plant}$ is the concentration of a metal in the tissue of a plant and $[M]_{soil}$ is the total concentration of the same metal in the soil where this plant was grown (Pascual *et al.*, 2004).

RESULTS AND DISCUSSION

Chemical Properties

The characteristics of the soil, TSC and the experimental mixtures are given in Table 1. The pH of the TSC is slightly higher than the soil pH and it represents alkali conditions. On the other hand, the OM content of the TSC was as high as 38.29% which elevated the OM ratio in the experimental pots due to the increasing compost ratio in the experiments. The salinity of the TSC was high in concentration (22551 mg kg^{-1} Cl) where this value was determined as 656 mg kg^{-1} Cl for the soil sample.

Table 1. Chemical Properties of Soil, TSC, and the Mixtures.

Parameters	TSC	Soil (Control)	5% TSC	10% TSC	25% TSC	50% TSC
pH	8.30	7.42	7.52	7.62	7.75	7.95
Water content (%)	23.70	31.76	30.96	30.52	29.27	27.30
Organic matter (% dry weight)	38.29	27.64	28.18	28.82	30.64	32.54
Salinity (mg kg^{-1} Cl)	22551	656	2319	3007	5054	10012
Cr (mg kg^{-1})	57307.0	112.0	3040.0	7475.0	13674.0	29580.0
Cu (mg kg^{-1})	32.0	15.6	16.4	17.2	19.3	23.4
Zn (mg kg^{-1})	25.4	185.2	182.3	171.9	145.5	105.6
Ni (mg kg^{-1})	36.2	73.2	70.4	68.2	65.6	42.4

The Cr concentration in the TSC was remarkably high as 57307 mg kg^{-1} due to the sludge coming from the treatment plant of the tanning process. The Cr value of the soil was detected as 112 mg kg^{-1}. The high Cr concentration in the TSC significantly increased the Cr levels in the mixtures. The other metals were found in lower concentrations such as Cu 32 mg kg^{-1}, Zn 25.4 mg kg^{-1}, and Ni as 36.2 mg kg^{-1}. In the soil, Zn concentration was detected as 185 mg kg^{-1} which is higher than the level in TSC. The same situation was observed for Ni which was detected as 73.2 mg kg^{-1} in soil. These lower Zn and Ni concentrations in the TSC decreased the levels of these metals in the experimental pots.

Plant Growth

The germination of the GSM and Ecopalmix types started rapidly on the 6th day after sowing in the control and in 5% TSC pots, while germination in the pots having 25% TSC started on the 8th day. The 50% TSC carrying pots gave the shoots of GSM and Ecopalmix types on the 22nd and 24th days of the planting. The developments of the plants in these pots were relatively slow. Pimpernel started to shoot slowly, after the 9th day of the planting, but only in control, 5% TSC, and 10% TSC pots. No development could be observed on the other pots for this single specie. The harvested plants were combined and the ratio of the shoot mass in the mixtures (G_i) to the shoot mass in the control sets (G_c) are presented with Figure 1. Here, the 5% TSC and 10% TSC application to the soil enhanced the growth of the Ecopalmix and GSM types while 25% TSC mixtures did not show significant mass changes when compared to the control set. These results showed that, under certain mixing conditions, the mix-seed grass types show tolerance to the tannery sludge compost. On the other hand, 50% TSC application disrupted the growth of the mix-seed grass types. Besides, no growth enhancement in the Pimpernel grass could be observed with TSC application to the soil. This may depend on the sensitivity of this single specie. The results of 25% and 50% TSC application could not observed and reported for Pimpernel, since there was no germination in those pots.

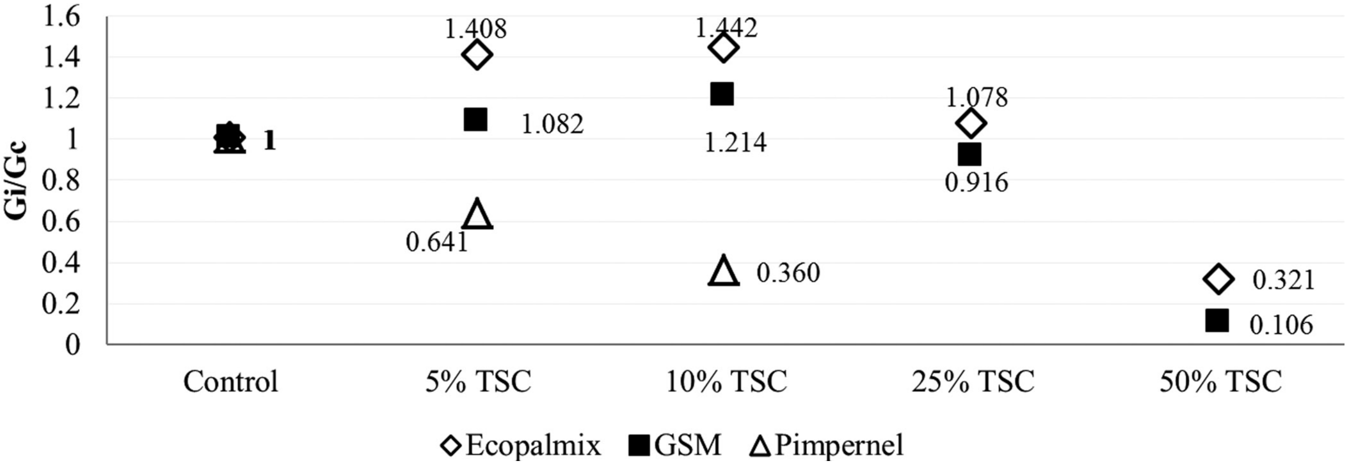


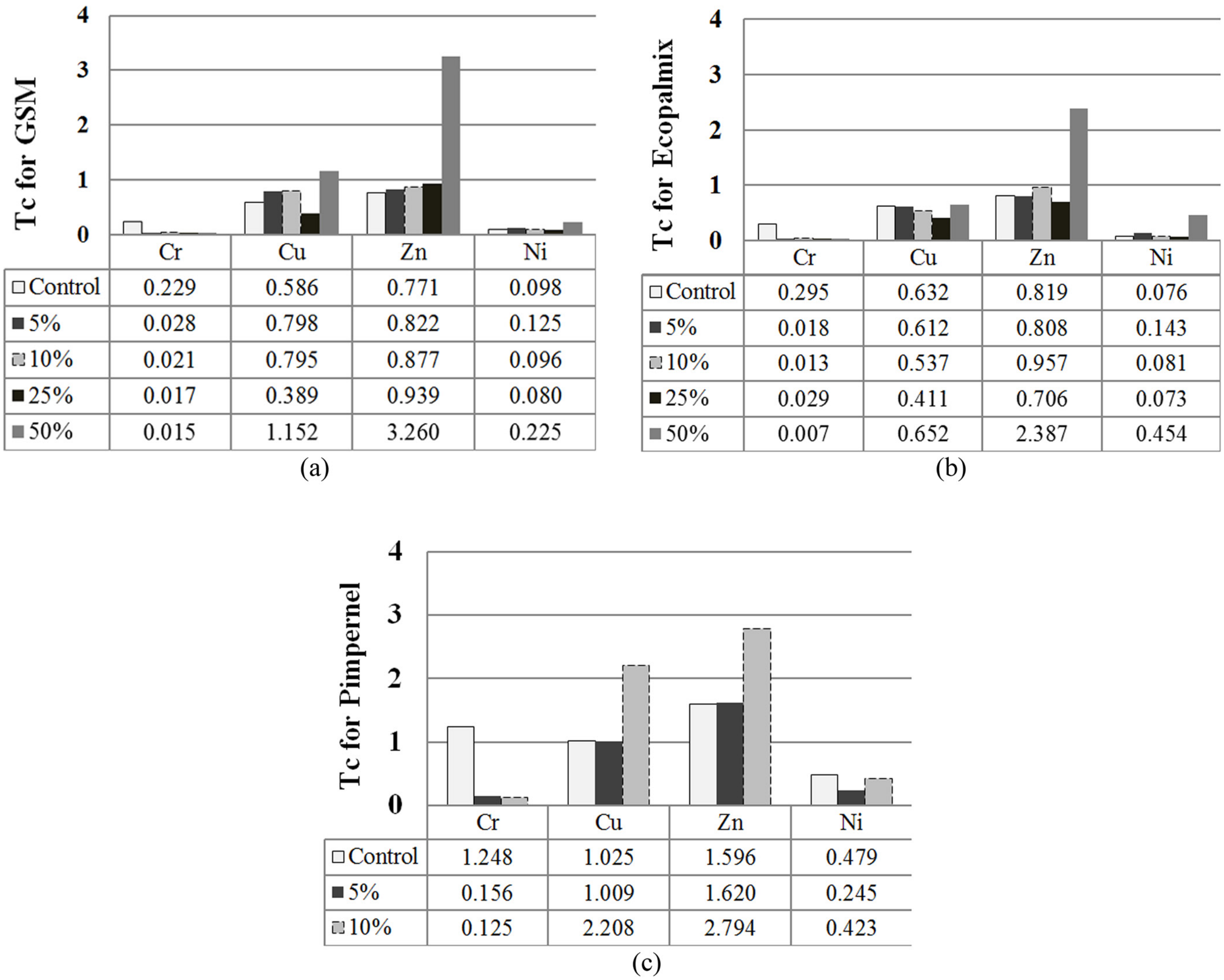
Figure 1. *The ratio of the plant masses in the mixtures to the control pots (G_i: plant mass in the mixtures, G_c: plant mass in the control pots).*

Heavy Metals in Plants and Transfer Coefficients

The concentration of metals in the dry matter of grass shoots are summarized in Table 2. The increasing addition of TSC remarkably increased the Cr concentrations in the plants. This is can be explained with the significant concentration elevations in the soil mixtures depending on the extreme Cr level in the compost. On the other hand, no trend in the Cu, Zn, and Ni concentrations were observed in the plants due to the increasing TSC ratios. Random concentrations were detected in the 5%, 10%, and 25% TSC trials for GSM and Ecopalmix. However, the highest Cu, Zn, and Ni concentrations were determined in the plants grown in 50% TSC for these types. As mentioned previously, Zn and Ni concentrations in the soil mixtures decreased due

Table 2. Plant Heavy Metal Concentrations in Different Experimental Pots (mg kg^{-1}, dry weight).

		Cr	Cu	Zn	Ni
GSM	Soil (Control)	25.71	9.14	142.56	7.14
	5% TSC	83.90	13.08	148.43	8.82
	10% TSC	154.14	9.50	149.98	6.53
	25% TSC	233.38	7.39	136.22	5.28
	50% TSC	426.30	26.95	641.90	9.45
Ecopalmix	Soil (Control)	33.13	10.07	151.54	5.58
	5% TSC	54.72	10.03	147.14	10.03
	10% TSC	98.78	9.13	163.65	5.54
	25% TSC	392.40	7.80	102.30	4.80
	50% TSC	403.52	15.26	250.58	19.08
Pimpernel	Soil (Control)	140.22	15.99	295.20	35.06
	5% TSC	476.00	16.54	238.97	17.35
	10% TSC	935.15	37.54	277.82	28.67



	Cr	Cu	Zn	Ni
□ Control	0.229	0.586	0.771	0.098
■ 5%	0.028	0.798	0.822	0.125
▨ 10%	0.021	0.795	0.877	0.096
■ 25%	0.017	0.389	0.939	0.080
■ 50%	0.015	1.152	3.260	0.225

(a)

	Cr	Cu	Zn	Ni
□ Control	0.295	0.632	0.819	0.076
■ 5%	0.018	0.612	0.808	0.143
▨ 10%	0.013	0.537	0.957	0.081
■ 25%	0.029	0.411	0.706	0.073
■ 50%	0.007	0.652	2.387	0.454

(b)

	Cr	Cu	Zn	Ni
□ Control	1.248	1.025	1.596	0.479
■ 5%	0.156	1.009	1.620	0.245
▨ 10%	0.125	2.208	2.794	0.423

(c)

Figure 2. *Transfer coefficients (T_c) of heavy metals in shoots of the grass types in 0% (control), 5% TSC, 10% TSC, 25% TSC, and 50% TSC amendments.*

to the increasing TSC ratio, depending on the lower Zn and Ni concentrations in the compost. These results may be due to the binding forms of these metals to the soil mixtures in the pots, since the chemical distribution of the metals in the soils gives better indication of the metal availability. No complete results can be presented for Pimpernel since there was no plant growth in the 25% and 50% TSC pots. However, it should be noted that the Cr concentration in Pimpernel shoots are much higher than the Cr level in Ecopalmix and GSM in all trials, which may be the indication of higher uptake capacity of this plant.

Transfer coefficients (T_c) were also calculated here, to assess the metals availability. All the T_c values calculated here are in comparison with the T_c intervals reported by Kloke *et al.* (1994). In this study, Zn was the most mobile element, as it had greater T_c values than Cu, Ni, and Cr does (Figure 2). In all the grass types, the T_c values for chromium decreased as the TSC ratio in the mixture increased. This can be explained by the chemical binding form of Cr in compost, which may be very high in crystal matrix, and not available for plant uptake. On the other hand, the elevated TSC application rates led to greater T_c values for Cu, Zn, and Ni. These increasing T_c values for the mentioned elements may be due to the chemical fractionation of heavy metals in the mixtures. Cu, Ni, and Zn are probably present in more labile forms in compost, such as extractable, reducible, or oxidizable. The order of mobility according to the T_c values is Zn > Cu > Cr > Ni. This situation is in agreement with Antoniadis and Alloway (2001).

CONCLUSIONS

The findings of the current study can be listed as follows:

- 5% TSC and 10% of tannery sludge application to the leaf mould soil enhanced the growth of the Ecopalmix and GSM types while 50% TSC application disrupted the growth of the mixed grass types.
- Similar evidences were observed for commercial GSM and Ecopalmix grass types, since the content of these grasses were approximately similar.
- The compost addition considerably increased the Cr concentrations in plants due to the extreme Cr level or the binding forms of this element in the compost. The highest Cu, Zn, and Ni concentrations were determined in the plants grown in 50% TSC for these types. This may depend on the chemical distribution of these metals in the TSC mixtures.
- The order of mobility according to the T_c values was Zn > Cu > Cr > Ni.

It can be concluded that under certain conditions heavy metals containing sludge compost may be applied to soils. However, the chemical binding forms of the metals must be determined and presented to prevent the subsurface migration of their labile forms.

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Effects of Wastewater Sludge Amendments on Soil Enzyme Activities in Earthworm Cast

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ABSTRACT: Land application of sludge needs to be properly managed to avoid the detrimental effects on micro and macroorganisms in the soil. Addition of sludge to the soil environment may impact earthworm activity, and in turn, soil productivity and health. In this study, two wastewater sludges which were different in origin (municipal and canned-food industry) were added to soil microcosms containing earthworms at different application rates. Alkaline phosphatase, dehydrogenase, β-glucosidase and urease activities were measured in the earthworm casts collected from sludge-amended and control pots throughout a 30 day incubation period at 20°C. These results suggest that sludge origin was the primary factor that influence soil ecosystems.

INTRODUCTION

SOIL ENZYMATIC ACTIVITIES are commonly used as biomarkers of soil quality. Several organic and inorganic compounds found in wastewater sludges make them possible to reuse as fertilizer. Monitoring and evaluating the quality of sludge amended soils with enzyme activities accepted as a beneficial practice with respect to sustainable soil management. Soil enzymatic activity assays act as potential indicators of soil quality being operationally practical, sensitive, integrative. Besides, they are measures of the soil microbial activity and therefore they are strictly related to nutrient cycles and transformations [1,2]. Moreover, as claimed by several authors, [3,4,5,6] soil enzyme activities may be considered early and sensitive indicators of soil alteration in both natural and agro-ecosystems, thus being well suited to measure the impact of pollution on the quality of soil.

The enzyme levels in soil systems vary in amounts primarily due to the fact that each soil type has different amounts of organic matter content, composition and activity of its living organisms and intensity of the biological processes [7]. Especially, earthworms play important roles in soil organic matter dynamics by regulating mineralization and humification processes [8,9] and improving physical and chemical properties of soil [10,11]. Earthworms have been demonstrated as indicators of anthropogenic land use. Lumbricus terrestris is one of the most important species of earthworm in temperate agroecosystems because of its abundance and ability to incorporate large amounts of surface litter into the soil surface [12].

In practice, the biochemical reactions are brought about largely through the catalytic contribution of enzymes and variable substrates that serve as energy sources for micro-organisms [13]. These enzymes may include amylase, arylsulphatases, β-glucosidase, cellulose, chitinase, dehydrogenase, phosphatase, protease and urease released from plants [14], animals [15], organic compounds and micro-organisms [16] and soils [17].

β-glucosidase is characteristically useful as a soil quality indicator, and may give a reflection of past biological activity, the capacity of soil to stabilise the soil organic matter, and can be used to detect management effect on soils [18].

Dehydrogenase enzyme is known to oxidise soil organic matter by transferring protons and electrons from substrates to acceptors. These processes are part of respiration pathways of soil micro-organisms and are closely related to the type of soil and soil air-water conditions [19]. Since these processes are part of respiration pathways of soil microorganisms, studies on the activities of dehydrogenase enzyme in the soil is very important as it may give indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility.

In soil ecosystems, phosphatases are believed to

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play critical roles in P cycles [20] as evidence shows that they are correlated to P stress and plant growth. Apart from being good indicators of soil fertility, phosphatase enzymes play key roles in the soil system [21].

Urease enzyme is responsible for the hydrolysis of urea fertiliser applied to the soil into NH_3 and CO_2 with the concomitant rise in soil pH [22]. This, in turn, results in a rapid N loss to the atmosphere through NH_3 volatilisation [23]. Due to this role, urease activities in soils have received a lot of attention since it was first reported by Rotini [24], a process considered vital in the regulation of N supply to plants after urea fertilisation.

The objectives of this study was to determine the effects of different doses of wastewater sludges on enzyme activities (Alkaline phosphatase, β-glucosidase, Dehydrogenase, Urease) in earthworm casts.

MATERIALS AND METHODS

Materials

Surface soil samples (0–20 cm) were taken from an experimental station in the campus of Uluda? University. The site is located in the Marmara Region, Northwestern Turkey (Latitude, 40°15′N; longitude, 28°53′E). The soil used in this experiment contained 62.3% clay, 14.5% silt and 23.2% sand (clay soil). Soil texture can accordingly be classified as an order of “entisol” and suborder of “fluvent” [25]. The soil chemical properties are shown in Table 1.

The canned food sludge samples (FWS) were obtained from a canned food company treatment plant in Bursa, Turkey that uses a flow rate of 5500 $m^3 \cdot day^{-1}$ to treat wastewater. The sludge was aerobically digested as a mixture of primary and waste activated sludge entered the digester. Primary and thickened biological sludges were dewatered with a belt press, and the fresh sludge cakes were air-dried in sand beds. The amount of dried wastewater sludge was 45–50 ton/year. The municipal wastewater sludge sample (MWS) was obtained from the treatment plant of a municipal wastewater treatment plant in Bursa which treats wastewater at flow rate of 64.000 $m^3 \cdot day^{-1}$. General characteristics of the canned food sludge and municipal wastewater sludge are presented in Table 1. Earthworms were collected from the soil sampling field. The collected earthworms were washed with distilled water and were kept stored on wet filter papers for 24 h prior to starting the experiment. *Lumbricus terrestris* was used for this study because it is the dominant earthworm species temperate climate soils. *Lumbricus terrestris* is a large deep-burrowing earthworm that builds permanent vertical burrows. However, it mainly feeds on the organic materials found at the soil surface. *Lumbricus terrestris* strongly impacts the transformations of organic matter in soil and improves soil fertility [26].

Table 1. The General Characteristics of the Canned Food Industry and Municipal Sludge and the Soil are Presented.

	Value		
Properties	**FWS**	**MWS**	**Soil**
pH (1:5 deionised water)	6.58	6.22	7.76
$EC_{25°C}$ 1:5 deionised water, dS m^{-1})	3.28	3.65	0.161
Organic C, %	34.50	30.26	1.23
Total-P, %	0.90	1.65	0.17
Total-N, %	5.06	5.14	0.15
NH_4^+-N (mg kg^{-1} dry sludge)	201.93	122.27	29.63
NO_3^--N (mg kg^{-1} dry sludge)	171.65	20.38	19.75
Mineral-N (mg kg^{-1} dry sludge)	373.57	142.65	49.38
Zn (mg kg^{-1} dry sludge)	12.55	416	1.35
Cu (mg kg^{-1} dry sludge)	4.10	237	2.05
Ni (mg kg^{-1} dry sludge)	< 5.00	94	< 1.00
Cr (mg kg^{-1} dry sludge)	4.12	286	< 1.00

Experimental Procedure

Soil samples were air-dried in the laboratory and sieved through 2 mm screens. Next, 1000 g of soil were placed in 1 L cylindrical plastic containers. Wastewater sludges were thoroughly mixed with the soil at 20, 40 and 80 g kg^{-1} on dry weight basis. A control treatment without wastewater sludges were also included. After mixing, seven *L. terrestris* individuals were placed in the control and the sludge amended soil samples. This number was chosen based on previous studies that used the same species [27]. A completely randomised design was used for the incubation study, and each pot was replicated three times. The pots were incubated for 30 days in the dark at 20 ± 0.5°C. The moisture content of the soil was maintained at 70% of field capacity throughout the incubation period. Earthworm casts deposited on the soil surface were collected daily and stored separately during the incubation period. The dried cast samples were analysed at the end of incubation period.

Chemical Analyses

Sample extracts were collected by shaking the samples with distilled water at 1:5 (w/v). The electrical conductivity ($EC_{25°C}$) and the pH of the sample extracts were measured with conductivity and pH meters, respectively. The nitrate and ammonium nitrogen concentrations were determined by steam distillation with MgO and Devarda alloy in samples that were extracted with 2 M KCl. The Kjeldahl digestion method was used to measure the total nitrogen concentration [28]. In addition, dichromate oxidation was used to measure total organic carbon [29].

The alkaline phosphatase (APA), dehydrogenase (DHA), β-glucosidase (BGA) and urease (UA) enzymatic activities were determined by using the methods described by Tabatabai [30] (with some modifications). Briefly, the alkaline phosphatase activities were determined by adding the modified universal buffer (pH = 11) and 0.025 M toluene and p-nitrophenyl phosphate solutions to the soil. The samples were then incubated at 37°C for 1 hour. The released p-nitrophenol (PNP) was quantified with a spectrophotometer at 410 nm. The dehydrogenase activity was determined by adding a triphenyl tetrazolium chloride solution (3%) to soil samples prior to incubating the suspensions at 37°C for 24 hours. The formation of TPF (triphenyl formazan) was determined with a spectrophotometer at 485 nm. The β-glucosidase activity test was also based on the colorimetric determination of PNP. The PNP released by the enzyme when the soil was incubated with buffered p-nitrophenyl-β-D-glucoside (PNG) and toluene solutions was measured. Similarly, the assay for urease activity was based on the determination of NH_4^+ that was released by urease when the soil was incubated with the THAM buffer (pH = 9), a 0.02 M urea solution and toluene at 37°C for 2 hours. The formation of ammonium was determined by steam distillation.

RESULT AND DISCUSSION

Wastewater sludge seems to stimulate soil enzyme activity by increasing the available carbon, nutrients and/or microbial activity [31,32,33]. Statistical results of this study revealed that enzyme activities in cast were significantly dependent on sludge doses and sludge type ($p < 0.01$). Variations of dehydrogenase activity (DHA) levels in sludge amended casts at the end of incubation period of 30 days are shown in Figure 1.

The dehydrogenase activity levels were apperantly higher in canned food industry sludge amended cast than those in the control cast ($p < 0.001$). The greater dehydrogenase activity noted that the added waste did not include compounds which were toxic for this activity [34,35] or that the increase due to microbial growth (with the consequent increase in the enzyme activity) and/or addition of microbial cells or enzymes with the amendment with organic materials, might have counteracted any inhibitory effect by toxic compounds [36,37]. The highest DHA activity in cast was obtained with the FWS application dose of 20 g kg^{-1} after the incubation period of 30 days. Higher doses of sludge amendment did not lead to higher levels of activity. Application of higher amounts of sludge probably resulted in the accumulation of toxic metabolites which may have reduced earthworm activity.

The addition of municipal wastewater sludge significantly decreased DHA in cast. The studied sludge contains a certain amount of heavy metals (Table 1). DHA may be affected by sludge-derived heavy metals even for the all sludge dose [38]. Increasing sludge application doses are inclined to produce decreasing DHA levels in cast.

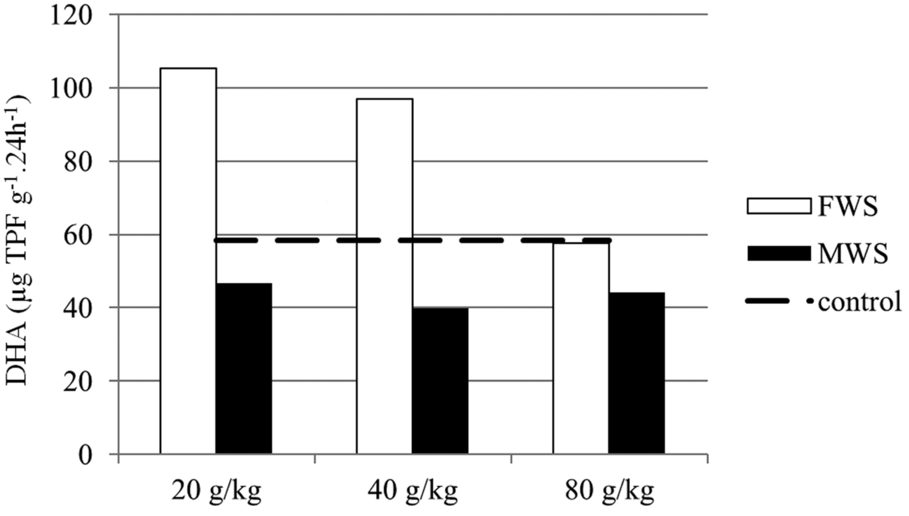

Figure 1. *Changes in dehydrogenase activity in amended casts at the end of incubation period.*

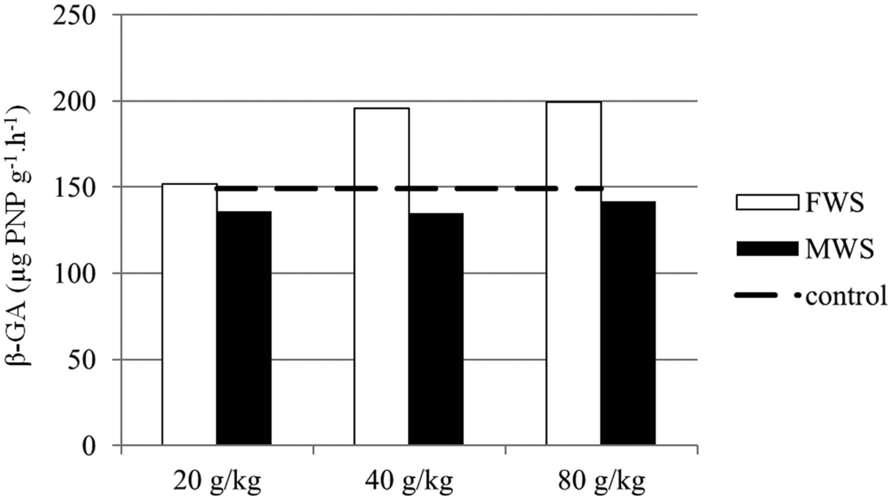

Figure 2. *Changes in β-GA in amended casts at the end of incubation period.*

Figure 2 shows the variation of β-GA in casts at different concentrations of the wastewater sludges. In this study, the cast collected from FWS amended soils showed significantly-higher β-GA than the control cast ($p < 0.001$), in agreement with other reports on the enhancement of hydrolytic enzymes by organic amendments [39,40]. The maximum β-GA level in casts collected from the soil amended with FSW was obtained with application of 80 g kg^{-1} at the end of incubation period. Level of β-GA in casts slightly increased with increasing sludge dose. The presence of a high content of degradable organic compounds (available substrate) in the wastewater sludge might have stimulated enzyme activity: as the substrate increased, the enzyme activity increased as well [41,42].

Levels of β-GA in cast samples were negatively affected by the MWS sludge amendments and no significant variation was determined in β-GA levels depending on sludge doses.

Figure 3 depicts the variation of urease activity in casts. The activity values in casts were significantly higher than control values for all sludge doses ($p < 0.001$). This is likely due to the activation of urease that results from increased organic carbon and nutrient content [33,43]. It was found that UA levels in casts showed an increasing trend with the increase of sludge doses. The highest levels were obtained with the application dose of 80 g kg^{-1}. In addition, the results indicated that UA levels in the casts from the FWS amended pots was generally higher than those in the casts from the MWS amended pots.

Variations in phosphatase activity, apart from indicating changes in the quantity and quality of soil phosphorated substrates [44,45], are also good indicators of

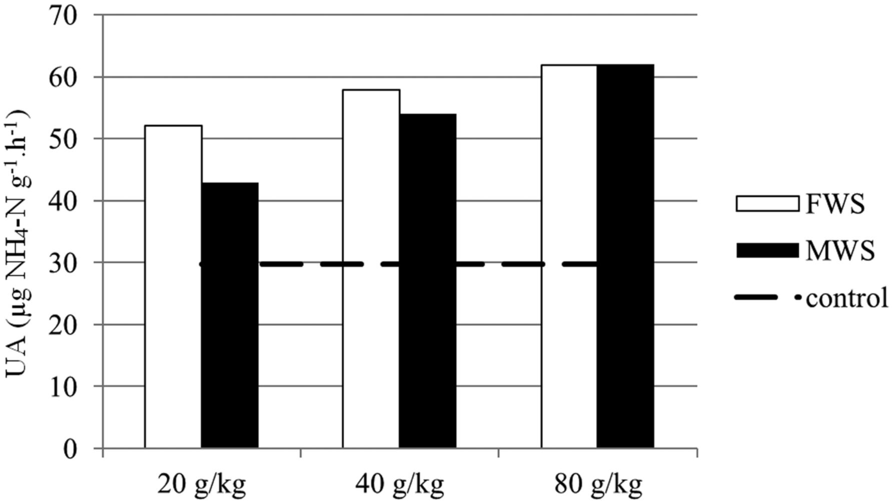

Figure 3. *Changes in UA in amended casts at the end of incubation period.*

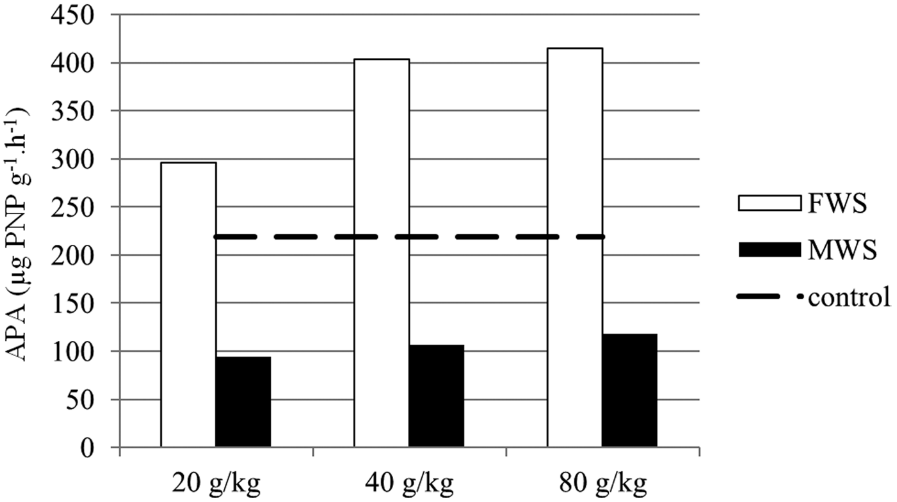

Figure 4. *Changes in APA in amended casts at the end of incubation period.*

soil biological status. Variations of alkaline phosphatase activity (APA) levels in cast at the end of the incubation period of 30 days are shown in Figure 4.

Canned food industry wastewater sludge (FWS) application significantly increased APA in casts for all treatments ($p < 0.001$). This increment in activity levels is probably related to the utilization of organic P [46,47]. APA levels in cast increased with increasing sludge doses ($p < 0.001$). Statistical results revealed that the APA in cast were significantly dependent on sludge doses and sludge type.

Contrary to FWS amendments, the APA levels in casts were negatively affected by the addition of municipal wastewater sludge (MWS).The measurud activity levels were significantly lower than those of control casts indicating the possible inhibition effect of MWS on APA.

When the sludge dose-dependent variation was evaluated, it was found that APA levels in casts slightly increased with increasing MWS dose. The dose-dependent increment was more pronounced in case of FWS.

CONCLUSIONS

The results of the study showed that canned food industry wastewater sludge amendment apparently increased urease, dehydrogenase, alkaline phosphatase and β-glucosidase activities in soil by 75–107%, 0–80%, 35–89% and 2–33%, respectively. The higher organic matter and lower heavy metal content of the canned food industry sludge appeared to be a more valuable food source for earthworms. The application of 80 g kg^{-1} of canned food industry sludge was the most effective application with respect to the enhancement of enzyme activities (except DHA) in earthworm casts. DHA level in sludge amended soil was similar to that of control soil.

On the other hand, municipal wastewater sludge application did not result in an increase in the soil alkaline phospatase, dehydrogenase and β-glucosidase activities in comparison to control levels. Urease activity levels in casts from MWS amended pots were enhanced by 44–108%, indicating that this activity was less sensitive to inhibition by sludge amendment than the other investigated enzyme activities.

The overall evaluation of the study suggests that sludge origin and application doses were the primary factors that influence functions of soil ecosystem. When the land application alternatives of wastewater sludges are evaluated, it will be important to investigate the biochemical transformation processes as well as functions of micro and macro organisms by pre-incubation studies.

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Technical, Economic, and Environmental Assessment of Wastewater and Sludge Management Solutions Designed to Overcome Common Issues

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ABSTRACT: In order to support decision-making in design of wastewater and sludge management within the European Union, the EU FP7 project ROUTES has performed technical development aimed at sludge minimisation, the enabling of agricultural use of sludge and sludge resource recovery. Technical, economic and environmental assessment has been performed for ten case studies in which model (non-existent) reference plants of different size and configuration, representative of real wastewater treatment plants in Europe, have been considered to be upgraded to solve different common problems, based on the new knowledge from the technology development carried out within the project. This paper reports on the methodology used in the assessment with examples of obtained results for three of the case studies. The methodology in particular highlights some critical points that need further attention when similar upgrading is considered in real cases.

INTRODUCTION

WASTEWATER and sludge management schemes in Europe are not always designed to optimally fulfil recent targets for wastewater and sludge management. Resource limitations and climate change are examples of increasing environmental pressures that make policy-makers push for resource recovery, e.g. in terms of energy recovery by incineration or utilisation of nutrients in sludge by applying sludge on agricultural land. As landfilling of sludge is being abandoned, inertisation and minimization efforts are of increasing interest for sludge of poor quality, for remote locations or for small plants. In order to support decision-making in design of wastewater and sludge management within the European Union, the EU FP7 project ROUTES has performed technical development aimed at sludge minimisation, at enabling of agricultural use of sludge and at resource recovery. To shed light on different consequences of the proposed upgrading, an integrated technical, economic and environmental assessment has been performed for ten case studies in which non-existent model reference plants of different size and configuration were considered to be upgraded to solve different common problems. This paper reports on the methodology used in the assessment with examples of the results obtained from this assessment for three of the case studies.

METHODOLOGY

An integrated approach for technical, economic and environmental assessment was developed and applied within the ROUTES project. In short, the methodology used for the assessment of technical, economic and environmental features was inspired by systems engineering [1,2], using an iterative approach involving several loops of definition and redefinition of systems, data collection and performance assessment. In practice, this was done in two major consecutive cycles—the first one providing input to modifications of technologies and of assessment methodology [3], halfway through the ROUTES project, and the second and final one, being partially reported on and discussed here, targeting primarily different decision-makers in the European Union (e.g. wastewater treatment plant (WWTP) operating companies and policy-makers).

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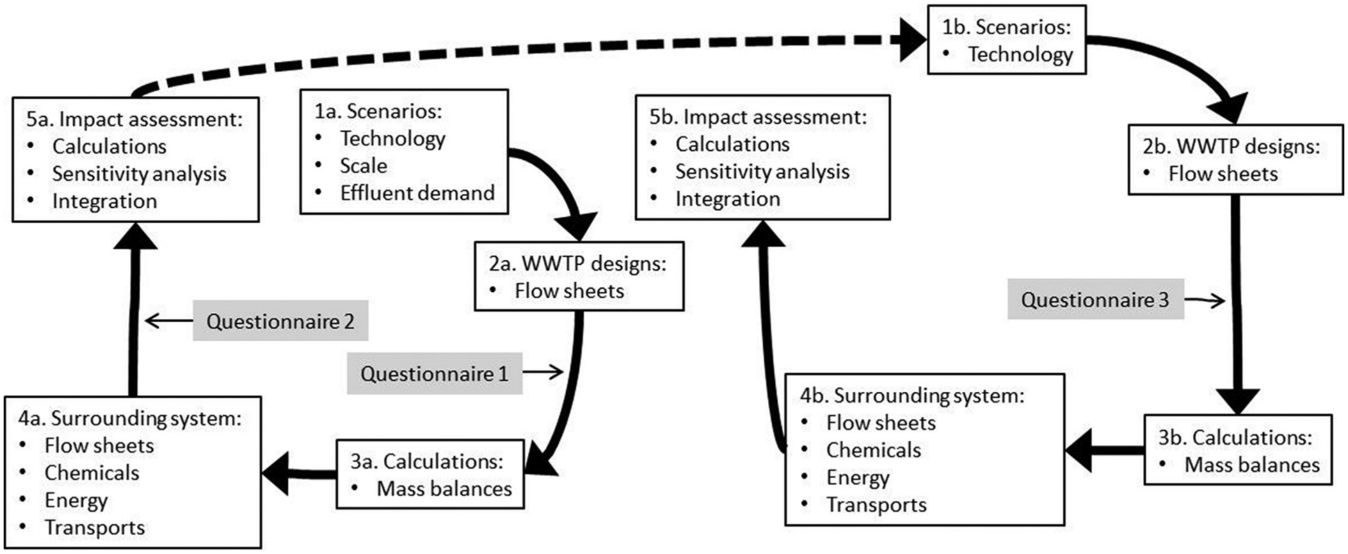

Figure 1. *The two cycles (1a-5a and 1b-5b, respectively) in the integrated assessment approach developed and applied within the ROUTES project.*

Figure 1 describes the overall activities in the two assessment cycles.

As a first step in the evaluation of technical, economic and environmental consequences of different types of upgrades of typical WWTPs, ten different case studies were outlined. Each case study consisted of a model (non-existent) reference WWTP considered to experience different types of common problems and at least one upgraded model plant in which different new technologies and designs had been considered to be introduced to solve these problems. As better detailed elsewhere [4], these case studies differ in terms of treatment capacity, target effluent quality, presence of primary settling and type of sludge stabilization (i.e. aerobic or anaerobic). Table 1 lists important features of reference plants and upgraded solutions, together with the scale of experimentation carried out within the ROUTES project. Among typical problems related to plant operation and sludge management, the case studies selected in project ROUTES focused on: (1) poor sludge quality (due to heavy metal and organic contamination); (2) poor sludge stabilization (due to incomplete stabilization and sanitation); (3) excessive nitrogen load from returning streams; and (4) underloaded anaerobic digester. These problems force the adoption of different sludge disposal options, based on the anticipated problems as well as on plant size and configuration. Hence, the different technical solutions proposed within the ROUTES project can be grouped according to their aim:

- sludge minimization in the water line; disposal option is still incineration but volumes are smaller (typically adopted for small and medium sized plants);
- improved sludge quality and resource recovery; new disposal option is agricultural use for nutrient recovery; different energy recovery measures are introduced (e.g. combined heat and power (CHP));
- intensive sludge minimization; disposal option is partly landfilling and partly agricultural use.

The methodology involves a preliminary design of WWTPs based on detailed plant-wide mass and energy balances, as described in Bertanza *et al.* [5]. Thereafter, the necessary details of the surrounding system, including transport distances, chemicals used and the use of different products were decided on.

Slightly different approaches were applied in the different parts of the assessment. In the techno-economic assessment, the consequence of changing from a reference scenario into a new scenario was assessed and results therefore represent the difference between the performance of the new solution and of the reference plant. The environmental assessment, however, aimed at also revealing dominant activities in the whole life cycle, and therefore, also the features that remained the same after the considered change were included. The environmental assessment also studied a broader system in terms of life cycle stages as it was performed using a life cycle perspective. The different aspects that were studied in the different parts of the assessment are listed in Table 2.

The technical evaluation focused on the role of the operator of a wastewater and sludge management facility and each main aspect contained several different subcategories. The methodology also contains notes on what type of data that is appropriate for each subcategory, e.g. data from research activities or from plant monitoring, and site-specific or generic data [5]. Data collected was filled into work sheets that had been prepared for calculations and the results were then analysed. The final results were eventually expressed by a traffic light type colour code [5], thus avoiding numerical values, and the discussion focused on highlighting aspects that need attention in real case implementation.

For the economic evaluation, cost items were calculated based on data derived from mass and energy balances. Calculated capital and operating costs refer to average loading conditions, which were considered to correspond to actual (design) loading conditions. The economic comparison was carried out by calculating the cost difference (gap) between the new and the reference solution. Since cost items are variable and depend on local conditions, a sensitivity analysis was performed in order to reveal critical factors. This was done by calculating variations in the final result assuming either the most favourable or the worst economic condition for all cost items.

The environmental assessment was performed as a life cycle assessment (LCA), following the international standards ISO 14040:2006, ISO 14044:2006 and the International Life Cycle Data Systems (ILCD) Handbook [6]. Environmental impacts from wastewater and sludge treatment as well as the sludge end-of-life, and the production of input materials such as electricity, heat and chemicals and sludge transports were included in the studied system. Wastewater collection was, however, not included. Environmental consequences of construction of the WWTP and of the processes added in the new solutions was not included as the environmental impacts of the assessed kind has been shown to typically be small for this part of such systems [7,8]. In cases where a marketable by-product was generated, a substitution approach was applied, giving the studied system benefits for the by-products by accounting for avoided production of a similar conventional product. The Gabi software from PE International was used for modelling the systems and the methods recommended

Table 1. Summary of Upgrading Objectives, Anticipated Problems and Proposed Technical Solutions for the Case Studies Analysed within the ROUTES Project.

Upgrading Objective	Anticipated Problem	Technical Solution	Source of Data
Sludge minimization in water line	Poor sludge quality	Sequencing Batch Biofilm Granular Reactor (SBBGR)	Pilot scale
		Membrane Bioreactor (MBR) with anaerobic side-stream reactor	Lab scale
		Alternated oxic-anoxic cycles in secondary treatment and sludge side-stream treatment	Full scale
Improved sludge quality and resource recovery	Poor sludge stabilization	Separate sludge dynamic pre-thickening, aerobic treatment after anaerobic digestion, pasteurization and CHP	Lab scale
	Excessive nitrogen load from returning streams	Ammonia stripping from returning streams and from source separated urine, and sludge pasteurization	Full scale
	Incomplete sludge processing	Sludge pre-treatment for improved rheology and digestibility, pumping to centralized plant and CHP	Pilot scale
	Poor sludge stabilization	Chemical and hydrodynamic hybrid disintegration and two-stage anaerobic digestion (mesophilic + thermophilic)	Lab scale
	Under-loaded anaerobic digester	Co-digestion with organic waste, CHP and composting Option with enhanced biological phosphorus removal and struvite recovery from digestate	Pilot scale
Intensive sludge minimization	Poor sludge quality	Dynamic sludge thickening, wet oxidation (WO), anaerobic digestion of liquid residue from WO, and CHP	Pilot and full scale
	Poor sludge quality; poor sludge stabilization	Separate processing of primary and secondary sludge: 1. Primary sludge treated by WO, anaerobic digestion of liquid residue, and CHP; 2. Secondary sludge treated by: • Dynamic thickening, sonolysis, 2-stage anaerobic digestion, and CHP; • Dynamic thickening, thermal hydrolysis, thermophilic anaerobic digestion, and CHP; • Dynamic thickening, anaerobic digestion, CHP and aerobic post-treatment	Lab, pilot and full scale

Table 2. Aspects Studied in the Technical, Economic and Environmental Assessment in the ROUTES Project (partly in Bertanza et al. [5] and modified since Svanström et al. [3]).

Main Technical Aspects	Cost Items	Environmental Impact Categories
Reliability of technology Complexity and integration with existing facilities Flexibility/Modularity Residues and recovered materials Consumption of raw materials and reagents Electric energy consumption Thermal energy consumption Energy available for external recovery Social & authorization aspects	Depreciation of new equipment Ordinary maintenance cost Cost of personnel Cost of electric energy Income from electric/thermal energy sale Cost for additional analyses for process control Cost of raw materials and reagents Income from recovered materials Cost for sludge disposal Cost of transportation	Global warming potential, GWP Acidification potential, AP Eutrophication potential for freshwater ecosystems, EP-F Eutrophication potential for marine ecosystems, EP-M Eutrophication potential for terrestrial ecosystems, EP-M Photochemical oxidant formation potential, POFP

in the ILCD handbook were used in the impact assessment.

Some results are shown in this paper mainly to illustrate what types of conclusions that can be drawn for different assessed upgrades. One technical solution has been chosen for each one of the upgrading objectives.

RESULTS AND DISCUSSION

In Table 3, results for the three case studies are reported in an overview form. When the difference between the reference and the upgraded system is less than 20% for an aspect, it has been judged to be similar. Some of the larger disadvantageous consequences of the upgrading are mentioned in the table. The assessment focused in particular on finding such concerns or large uncertainties. As many of the assessed aspects have been deemed to be highly site- and case-specific, any real case application must carefully look into all such issues. A careful check then needs to be made for the specific conditions that prevail in the specific case. In some cases, further technical development is needed.

In general, from the assessment results of all ROUTES solutions, it appears that most case studies exhibit only a few potentially critical points, which are heavily affected by local conditions. Critical issues relate to a large extent to the administrative and technical difficulties involved in upgrading the plant with the new technologies and to some extent to operational is-

Table 3. Examples of Results from the Integrated Technical, Economic and Environmental Assessment. Mainly the Type of Data that is Particularly Crucial to Check in Real Application of the Considered Upgrading is Reported on Here. For Evaluating the Feasibility of a Given Solution in Real Situations, and for a Complete Description of Features and Advantages, Documentation from All Relevant Parts of the ROUTES Project Should be Consulted (www.eu-routes.org).

Case Study	Technical Performance	Economic Performance	Environmental Performance
Upgrade of Membrane Bioreactor (MBR) with anaerobic side-stream reactor	The only potential concern is the reliability as there are only a few full scale applications and a large range of variability of process performance has been observed	No large differences; mainly influenced by depreciation and sludge disposal costs	No large differences; the slightly increased emissions from WWTP and for electricity generation are made up for by decreased emissions from sludge incineration
Upgrade with separate dynamic secondary sludge pre-thickening, aerobic treatment after anaerobic digestion, pasteurization and CHP	Only critical issue could be the additional work required for managing the energy recovery procedures and devices	Likely to be advantageous depending on local conditions; strongly influenced by sludge disposal costs and income from sale of electricity	Quite positive; increased content of phosphorus in WWTP effluent gives high eutrophication potential; some potential issues related to agricultural use have not yet been assessed (e.g. toxicity and ecotoxicity)
Upgrade with dynamic sludge thickening, WO, anaerobic digestion of liquid residue from WO and CHP [9]	Potentially critical issues could be related to the complexity of the new technologies (with respect to conventional WWTPs) and the consumption of reagents, in particular methane and pure oxygen	Likely to be advantageous depending on local conditions; strongly influenced by sludge disposal costs and costs for reagents	All assessed impacts turn out positively; the gain from heat recovery and decreased emissions from sludge disposal more than makes up for the increased use of chemicals

sues, e.g., the management of an increasing number or amount of chemicals. The economic assessment often provides a rather broad range in terms of the cost gap. This is because many parameters have been deemed to be highly case-specific. As the estimates have been made for model plants that do not exist in reality, the capital cost of the upgrade is an item that is typically connected to very large uncertainties. Nevertheless, the identification of the most relevant cost item allows to better define the pre-requisites for profitable application. For example, in case of MBR with an integrated side-stream reactor, the existence of spare volumes (e.g. decommissioned secondary settling tanks) could result in strongly reducing the impact of depreciation costs on the economic outlook of this solution. In general, it was found that the costs related to sludge disposal and to materials and reagents have a large influence on the economic outcome of most of the assessed scenarios. For the environmental assessment, most upgrades seem to be quite unproblematic. However, for a few systems, phosphorus emissions to water and ammonia emissions to air generate a disadvantageous situation. This indicates what to look out for in actual design in real cases.

In the assessment made in this project, several different upgrades are sometimes made in the same case study. This makes it difficult to distinguish the effects of one particular upgrade from another. The case studies were not designed to study separate technologies but rather for studying how problems at a WWTP can be solved by a combination of solutions. This has to be taken into account when results are interpreted.

As a general remark, just as anaerobic digestion seems to always be beneficial for keeping external energy requirements low, it is generally of large importance to keep the electricity requirements for different processes low. Sludge reduction or stabilisation can often be achieved to a satisfactory level, but this may come at the expense of an increased electricity use. However, improvements may be possible if this issue is highlighted, which it was in the ROUTES project because of the integrated assessment. In the first cycle of assessment activities, some technologies were hampered by a high electricity use. As this was highlighted in the assessment, this could be addressed and overcome by technical development to result in an acceptable performance in the second cycle of assessment.

As average European electricity mix was assumed for the environmental assessment in all cases (about 27% nuclear, 22% natural gas, 15% hard coal, 12% hydro etcetera), electricity use could, in other energy surroundings, be of lower or higher importance than found here. The electric energy generated with the introduction of CHP often makes up for an increased electricity use due to the intensive treatment adopted in most of the case studies considered in the ROUTES project [4]. Despite this positive contribution of CHP to the electric energy balance, its introduction is always associated with some additional difficulties in terms of technical operation.

The case studies that were explored in this project do not represent real plants but are model plants that simulate problems commonly experienced in real WWTP. In terms of experimentation, data was gathered from many different sources, ranging from laboratory to full scale and performed for both model and real wastewater and sludge. Data was also gathered from different partners in the project and from literature. The uncertainties associated with this procedure do not allow for a fully quantitative evaluation and the results have therefore been presented in a semi-quantitative manner, with focus on critical aspects that need special attention. These aspects must be considered when technologies are implemented in real cases, together with possible trade-offs that need to be considered, e.g., when an improved environmental impact comes at an economic cost, or similar. Our strong recommendation is that for each case in which these upgrades are considered for real cases, data should be checked and modified if needed. In the results reporting from the assessment, care has been put on noting which data that are particularly crucial to check for the specific case.

It can be argued that some potentially important parameters of technical, economic and environmental nature are lacking for a full understanding of all impacts. For example, as many of the case studies aim at improving the quality of sludge, rendering it suitable for agricultural use, some potential impacts that have not yet been assessed should be further evaluated, e.g. potential impacts related to the content of heavy metals, organic micro-pollutants and pathogens in sludge applied on land. Although first attempts were made to assess such impacts in the ROUTES project, it is still too early to quantify such effects, both because of a lack of accepted and well developed methodology and because of a lack of data. Further research is needed to push the frontier forwards in this respect. Note that there may also be considerable positive impacts of the use of sludge in agriculture that have not been assessed either, such as the increase of soil quality or carbon sequestration in soil. However, the integrated assessment methodology developed and applied, in general, does not focus on positive impacts but rather highlight issues of potential concern that need more careful evaluation.

The different parts of the assessment were not made in a strictly comparable way. There are differences in the inherent system boundaries in the different assessments as different types of stakeholders are concerned. The technical evaluation focuses on the role of the operator while the economic evaluation focuses on the cost for the companies that manage wastewater and sludge and the environmental assessment was made with a much broader scope, putting nature and man in general in focus and with a life cycle perspective. It can be argued that these broader considerations will, in the future, be internalized in economic evaluations as companies will be increasingly forced to pay a price for pollution and resource extraction that better represents values that are affected. However, as long as the three different sets of results are interpreted in light of the varying scope and still seen to bring important clues to the full understanding, this should be unproblematic. Furthermore, technical and economic evaluations in the applied methodology study only the parameters that change because of an upgrade while the environmental assessment attempts to cover the impact of all activities. The most important implication of including all activities is that a difference between the reference and the new plant may seem to be insignificant in relation to the whole while, in fact, it would seem much larger if only the change had been considered. This also needs to be taken into account in the interpretation.

CONCLUSIONS

The integrated technical, economic and environmental assessment methodology developed in the ROUTES project was successfully applied in ten case studies. Each of these cases evaluated the consequences of upgrading a model (non-existent) reference WWTP simulating problems commonly experienced in real treatment plants. In almost all case studies, the proposed upgrade turned out to have a positive outcome for most of the assessed aspects. The methodology allowed highlighting some critical points that need further attention when similar upgrading is considered in real cases. In many of the case studies, results were seen to be highly dependent on specific local conditions. Some important trade-offs were identified, e.g. when new technologies are introduced that will decrease the environmental burden, this may come at the cost of additional efforts for the operator and with large uncertainties in the economic outcome. The proposed methodology was deemed to provide valuable support in defining priorities for future wastewater and sludge management, as it is suitable for the evaluation of complex systems like the ones considered in the ROUTES project. When applied for assessing real cases, the large uncertainties seen when applying the developed methodology to model scenarios can be reduced by the use of case-specific data.

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Synthesis of Zeolites Using Paper Industry Sludge as Raw Material

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ABSTRACT: The objective of this study was to use sludge generated from the paper industry wastewater treatment plants as raw material for the synthesis of zeolites. Sludge samples were collected from the three main paper industries of the Metropolitan Zone of Mexico City and the one having the highest content of Si and Al was selected to conduct the zeolite synthesis tests. The selected sludge was calcined, leached with HCl solutions and used in the zeolites synthesis process. Sludge leaching with 1M HCl solution promotes the formation of zeolite A and 2 M HCl solution promotes zeolites P (calcium and sodium forms). The successful use of sludge generated from the paper industry in the synthesis process of zeolites will result in a reduction of the pollution associated to these residues. This represents important technical, economic and environmental advantages compared to other similar processes that use high purity commercial raw materials.

INTRODUCTION

The paper industry generates a significant amount of solid wastes requiring appropriate disposal. In 2010, 4 million tons of paper sludge was produced worldwide, with the United States of America being the largest generator and Mexico the eighteenth [1].

Sludge composition depends on the characteristics of the raw materials used to generate these wastes [1]. Generally, they consist of an organic fraction (cellulose fibers) and an inorganic fraction (minerals, such as kaolin, calcite and titanium oxide).

In order to reduce the volume of these wastes and because of their high organic matter content, they are usually dried, incinerated and used for soil improvement purposes or are disposed of in landfills [2]. However, several valorization options have also been investigated, such as the use of paper sludge ashes as aggregate in the building industry [3]. Paper sludge can also be used in manufacturing of adsorbent materials. In this case, organic material can be transformed to activated carbon through calcination and inorganic matter can be transformed into gehlenite, anorthite, and zeolites [4,5]. Adsorbing material products have been used for removing phosphates and methylene blue.

Table 1 shows examples of residues and kaolinite use for synthesis of zeolites and other adsorbing materials. The SiO_2/Al_2O_3 molar ratio ranges from 1.2 to 3.8, temperature varies from 40 to 200°C, and synthesis time ranges from 1 to 240 hrs. The most common values for the first two parameters are 2 (or very near this value) and 100°C, respectively. Reaction time varies widely and depends on the crystalline form of Si and Al compounds in the residue. However, average reaction time exceeds 8 hrs. Only Yaping *et al.* [6], used times ranging from 1 to 8 hrs, but in that study, an extraction of Si and Al was performed and then leached products were used in the zeolites synthesis process. In the majority of those studies, A-type and P-type zeolites were produced.

A-type zeolite ($Na_{12}Al_{12}Si_{12}O_{48}\ 27H_2O$) is used as a component in detergent formulation because of its important Ca^{2+} and Mg^{2+} removal capacity [15]. It is also used with high removal efficiency for capturing greenhouse gases such as CO, CO_2, and CH_4. It is also widely used in water treatment as an ion exchanger for sequestering Ca^{2+} and Mg^{2+} [15]. [RMRZ1]

P-type zeolite ($Na_8Al_8Si_8O_{32}\ 15.2H_2O$) is also used as a component in the formulation of detergents due to

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Table 1. SiO_2/Al_2O_3 Molar Ratio of Various Wastes for the Synthesis of Zeolites and Other Adsorbent Materials.

Used Waste	SiO_2/Al_2O_3 Molar Ratio	Synthesis Temperature, °C	Synthesis Time, h	Produced Zeolite	Reference
Fly ashes	1.89	100	5–13	A, P, X	[6]
Fly ashes	1.2–3.8	150–200	8–100	P, analcime, gmelinite, phillipsite.	[7]
Fly ashes	1.64	40–90	24	A, X, P, hydroxy-sodalite	[8]
Fly ashes	1.87	105	24	X, P, sodalite.	[9]
Fly ashes	3.26	40–120	2–72	P, HSOD, CHA	[10]
Spent catalytic cracking catalyst	1.86	80	2	A	[11]
Sludge treatment plant	3	63–86	18–48	A, P, X, sodalite	[12]
Kaolinite	2	100	1–240	A, X, Y	[13]
Kaolinite	1.94	70–80	1–8	A	[14]

A: zeolite A; P, Na-P1: type zeolite P, Na-P1; X: X-type zeolite; HSOD: hydroxy-sodalite; CHA: chabazite.

its Ca^{2+} and Mg^{2+} removal efficiency [16]. This zeolite is also utilized in the decontamination of wastewaters containing ammonium and heavy metals because of its high theoretical ion exchange capacity (7.37 meq/g). NaP-CaP-type zeolite is also used for simultaneously removing NH_4^+ and PO_4^{3-} from water, and thus Ca2+ can be substituted by Na+ through a reversible cation exchange reaction [17].

Based on aforementioned presentation, we proposed synthesis of A-and P-type zeolites at low reaction times and using paper industry sludge. Use of these wastes for zeolites production could have a dual benefit because, on one hand it contributes to mitigation of environmental footprint caused by accumulation and, on the other hand, zeolites can be used in wastewater treatment and for mitigation of atmospheric contamination caused by greenhouse gases.

MATERIALS AND METHODS

Sludge Sampling and Characterization

Sludge sampling was conducted in the wastewater treatment plants of three paper industries: *Grupo Mairo* (GM), *Kimberly Clark* (KC) and *Grupo Papelero Scribe* (GPS). The X-ray fluorescence technique (XRF) with a Siemens, SRS3000 equipment was used for the quantification of silicon (Si) and aluminum (Al) of the three sludge samples. Identification of the crystalline and mineralogical phases of these elements was conducted using the X-Ray Diffraction (XRD) method in a Bruker, D-8 diffractometer. The sludge having the highest Si and Al contents was selected. Identification of mineralogical phases for these elements helped determine best sludge conditioning processes when producing zeolites.

The experimental methodology for the zeolites synthesis using the selected sludge was divided into two stages: (1) conditioning of raw sludge sample and (2) application of the alkaline hydrothermal process to the conditioned sludge for synthesizing zeolites and (3) post-treatment of the produced materials. The first stage includes several processes: drying, calcination and leaching. The stages and processes involved are described hereinafter.

Sludge Conditioning Stage

Raw sludge was dried in an oven at 100°C during 24 hrs mainly for water evaporation purposes and thus for volume reduction. Afterwards, the dried sludge was calcined during 3 hrs at 550°C adapting the procedure proposed by NMX-AA-034-SCFI-2001 Water analysis—Determination of solids and salts dissolved in natural waters, wastewaters and treated wastewaters—Test method [18]. Calcination was carried out both for transforming kaolinite (zeolites precursor) in metakaolinite and for eliminating organic material. Metakaolinite is more reactive than kaolinite in the zeolite formation reaction [19].

Hydrochloric acid (HCl) solutions prepared at 1, 2 and 3 M were used in order to evaluate the effect of this reagent's concentration on the Ca, Si and Al leaching process and thus on the zeolites synthesis process. The ratio of sludge quantity: HCl solution volume was 0.1 g/mL. The leaching time was 24 hrs. At the end of this time, all samples were vacuum filtered in kitazato flasks; they were then washed with distilled water and dried at a temperature of 100°C for one hr. Once the conditioning stage of sludge was finished, zeolites were prepared using the alkaline hydrothermal method.

Zeolites Synthesis Stage

The solid generated in the conditioning stage was mixed with a 3 M sodium hydroxide (NaOH) solution in a 250 mL polymethylpentane flask. The solid/liquid ratio was 0.2 g/mL. The flask was heated under reflux with stirring at 90°C during 4 and 8 hrs.

At the end of these two reaction times, the mixture was filtered to recover the solid phase. The product was washed with 1 L distilled water at 80°C to remove NaOH residuals. Afterwards, it was dried at 100°C during 1 hr. Product was characterized using XRD and SEM.

RESULTS AND DISCUSSION

Paper Sludge Characterization through XRF and XRD

The main elements, quantified by XRF in terms of metallic oxides percentages, for the GM, KC and GPS sludges are shown in Table 2.

Three sludge samples contain Si and Al, which are the main zeolites precursors, but in different concentrations. The KC sludge is mainly formed by calcium compounds (CaO, 21.60%), silicon compounds (SiO_2, 21.33%), and aluminum compounds (Al_2O_3, 18.51%). The first of these elements could interfere with the zeolites synthesis process. The GPS sludge consists mainly of calcium compounds (CaO, 85.85%) and shows low Al and Si contents. With regard to GM sludge, besides the above elements, titanium (TiO_2, 25.38%) and iron (Fe_2O_3, 27.83%) are abundant. The concentrations of SiO_2 and Al_2O_3 obtained in this study are within the range of results reported for sludges generated by the paper industry worldwide [20–24]. Presence of calcium, silicon, and aluminum in the sludges of the Mexican paper companies results from additions of these elements as calcite and kaolinite during the paper making process. In the case of GM, the presence of titanium derives from the addition of TiO_2 during the cardboard making process. The presence of iron is due to ferric sulfate $Fe_2(SO_4)_3$ which is added as a coagulant during the coagulation/flocculation process in the wastewater treatment plant.

Table 2 also shows the chemical composition of other residues used in zeolites synthesis. On one hand, sludges from a wastewater treatment plant and slag ashes showed main element percentages very similar to the ones obtained for the paper sludge used in this work. On the other hand, variations were observed for all the compounds with respect to fly ashes, particularly SiO_2 (from 8 to 68%) and Al_2O_3 (from 5 to 35%). Rice husk ash showed the largest percentages of SiO_2 (94.1%) and the lowest of Al_2O_3 (0.2%). Despite these variations of Al and Si concentrations, zeolites synthesis was successfully achieved using these residues.

The SiO_2/Al_2O_3 molar ratio for KC, GM and GPS sludges was 1.87, 2.73 and 4.60, respectively. According to Barrer [25], the molar ratio of these compounds is appropriate for producing, among others, A- and P-type zeolites. GM and GPS samples showed a large amount of compounds that can interfere with zeolites synthesis. Moreover, calcination losses are very high (86%). Although the quantified SiO_2/Al_2O_3 molar ratio is within the range appropriate for producing zeolites, the paper sludge showed a significant quantity of organic material and calcium compounds, which can be disadvantageous in the zeolite synthesis process.

XRD analysis was conducted in order to identify the presence of kaolinite as main source of Si and Al in zeolite synthesis, and the type of other minerals present in the three sludge samples that could cause interference. Based on this information, the processes to be used for sludge conditioning prior to zeolite synthesis were determined. Figure 1 shows the diffractograms of the sludge samples dried at 110°C.

Table 2. Chemical Composition of the Main Elements of Three Paper Industry Sludges.

Sample	%SiO_2	%Al_2O_3	%CaO	%TiO_2	%Fe_2O_3	%P_2O_5	%MgO	%(Na_2O+K_2O+MnO)	%IL*	Reference
KC Sludge	21.33	18.51	55.73	1.60	0.53	0.41	1.85	0.35	53.43	This study
GM Sludge	12.71	7.90	21.60	25.381	27.83	2.58	1.176	0.80	86.55	This study
GPS Sludge	7.53	2.77	85.85	0.04	0.46	0.42	3.15	0.25	86.31	This study
HP Sludge	10.79	6.82	25.43	0.28	0.46	0.13	0.86	—	—	[20]
PTAR's Sludge	25	24	9	—	2	—	—	—	—	[21]
Flying ashes	8–68	5–35	2–37	—	1–32	—	1–27	—	—	[22]
Slag ashes	34.1	14.2	38	—	7.2	—	15	—	—	
Rice husks	94.1	0.12	0.55	0.05	—	0.06	0.95	0.5	1.82	[23]

*Ignition loss at 1000°C; HP: Holmen Paper, Madrid, Spain, WWTP= Wastewater Treatment Plant.

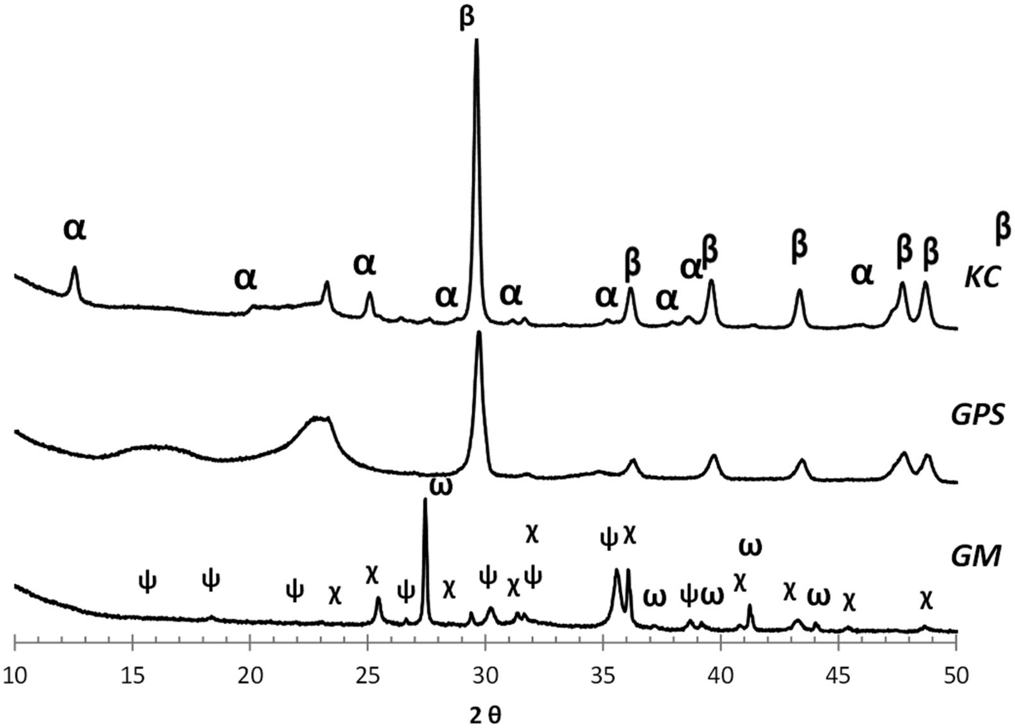

Figure 1. Diffractogram of three paper sludges: α kaolinite ($Al_2Si_2O_5(OH)_4$) (JCPDF# 05-0143), β calcite ($CaCO_3$) (JCPDF# 05-0586), ω Rutile (TiO_2) (JCPDF# 21-1276), χ Maghemite (Fe_2O_3) (JCPDF# 25-1402) and ψ Anhidrite ($CaSO_4$) (JCPDF# 72-0916). KC production of recycled paper, GP paper production and GM cardboard production.

In the GM and GPS samples, no crystalline and mineralogical phases of Si and Al were identified. In contrast, the KC sample, besides calcite, kaolinite ($Al_2Si_2O_5(OH)_4$) was identified. For this reason, the KC sludge was selected as the most appropriate for zeolites synthesis.

KC Sludge Leaching

The SiO_2/Al_2O_3 molar ratio calculated using the XRF results for the raw and leached KC sludges is shown in Table 3. It can be observed that the quantity of leached Al and the molar ratio increased with an augmentation in HCl concentration.

The application of a 1 M HCl solution to the sludge produced a leached product having a pH of 6.9. At this pH, aluminum can be found as $Al(OH)_3$, which is insoluble. This explains the low quantity of leached Al (2.1%). With regard to the sludges treated with 2 and 3 M HCl solutions, the leached Al percentage was higher (49.0 and 56.0%, respectively). In both cases, because the pH of the leached product was lower than 3, this element was present as the Al^{3+} soluble form [26], which explains the higher leached percentage in these last two cases compared to the percentage obtained with the 1 M HCl solution.

A large aluminum leaching will generate an increase of the SiO_2/Al_2O_3 molar ratio. Table 3 shows that only 2 and 3 M HCl solutions generated products with a SiO_2/Al_2O_3 molar ratio recommended for the synthesis of zeolites having high Al contents, such as A- and P-type zeolites.

Zeolite Synthesis by the Alkaline Hydrothermal Process (AHP)

Samples treated with a 4-hr AHP reaction time with 1, 2 and 3 M HCl solutions were coded A4, B4 and C4, respectively. Samples subjected to an 8-hr reaction time with 1, 2 and 3 M HCl solutions were denominated A8, B8 and C8, respectively. Figures 2 and 3 show

Table 3. Percentage of Aluminum Leached from KC Sludge with HCl.

HCl Concentration, M	Leaching pH	% of leached Al	SiO_2/Al_2O_3 Molar Ratio
KC*	–	–	1.88
1	6.9	2.1	1.91
2	0.31	49.0	3.67
3	–0.53	56.0	6.03

*KC sludge sample before HCl leaching.

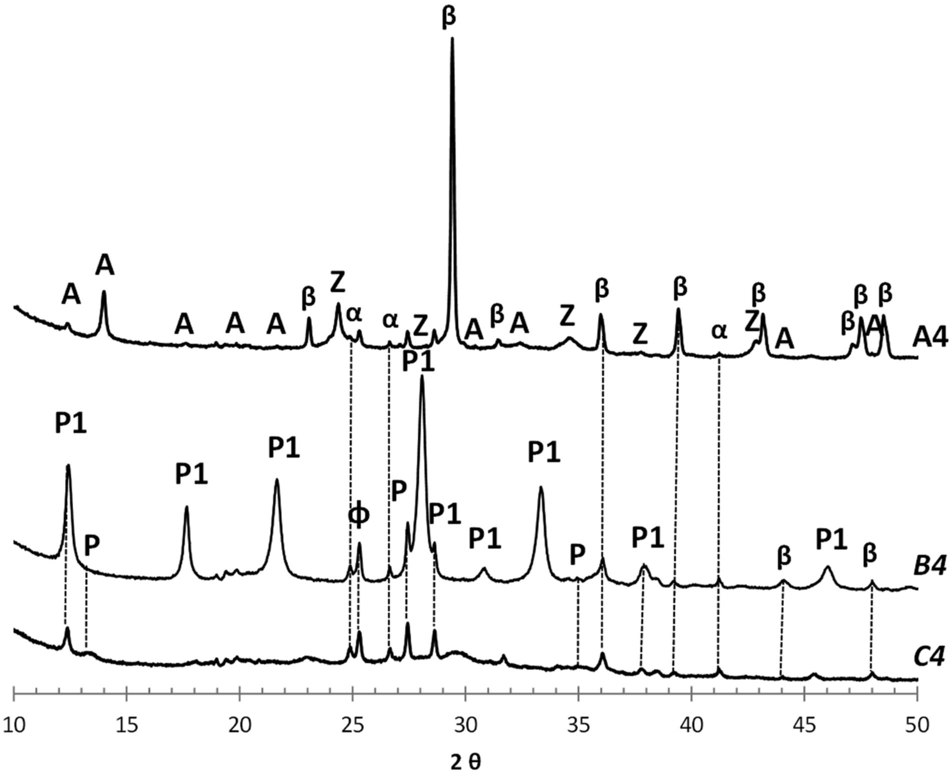

Figure 2. *Diffractograms of the samples synthesized through the alkaline hydrothermal method using a 4-hour reaction time. α, kaolinite ($Al_2Si_2O_5(OH)_4$); β calcite ($CaCO_3$); A A-type zeolite (JCPDF# 73-2340); P: NaP-CaP zeolite (JCPDF# 89-6321); P1: P-type zeolite (JCPDF# 39-0219).*

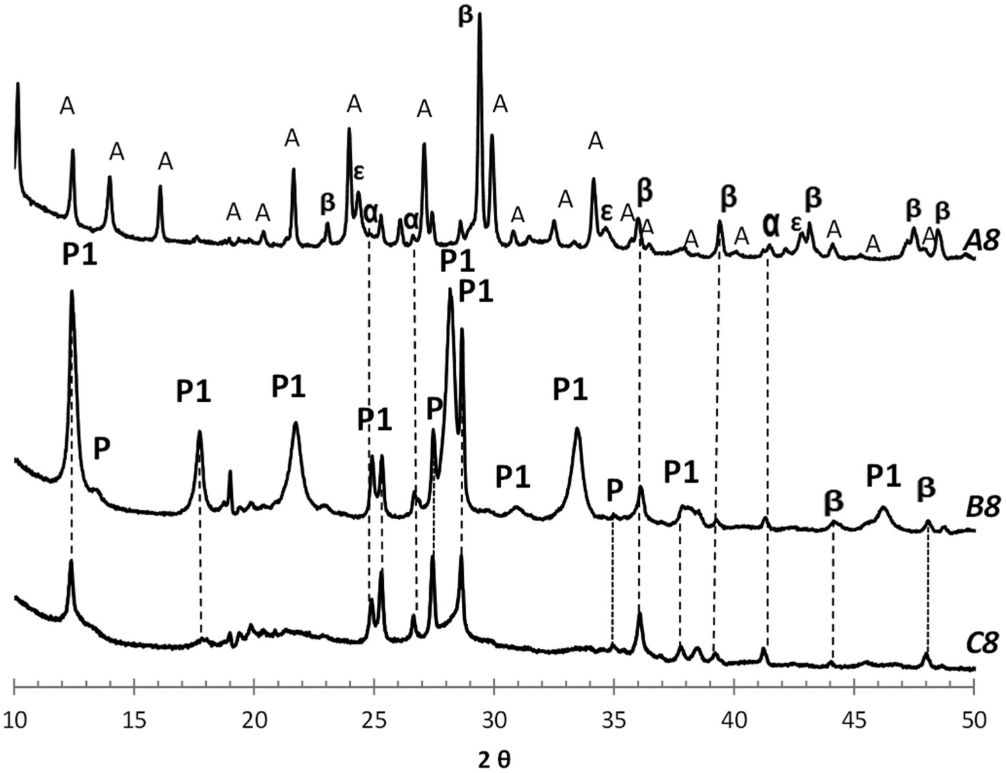

Figure 3. *Diffractograms of the samples synthesized through the alkaline hydrothermal method using an 8-hour reaction time. α, kaolinite ($Al_2Si_2O_5(OH)_4$); β calcite ($CaCO_3$); A A-type zeolite; ε zeolite, sodium aluminum nitrite hydrate formula $Na_8(Al_6Si_6O_{24})(NO_2)23H_2O$ (JCPDF# 47-0709); P: NaP-CaP zeolite; P1: Zeolite P.*

the diffractograms of the samples obtained with 4- and 8-hr reaction times, respectively.

In Figure 2, A- and P-types zeolite signals are observed. B4 sample shows the highest zeolite signal intensity compared to A4 and C4, nearly exclusively in the form of P-type zeolite. This means that, as estimated, a 2M HCl solution is adequate for obtaining a SiO_2/Al_2O_3 molar ratio for producing a P-type zeolite.

Calcite was identified in A4 meaning that a solution of 1 M HCl did not efficiently leach calcium. A4 and A8 (Figure 3) show signals of zeolite A, although in A8 these signals were higher than in A4. This showed that the formation kinetics of zeolite A was influenced by the presence of calcium probably because this element inhibits zeolite crystallization through the formation of calcium silicate, as reported by Barrer [25] and Juan *et al.* [27].

Six signals of calcite were observed in A4 and A8 samples. In contrast, only two of these signals were observed in B4, B8, C4 and C8 samples. This indicates a higher calcium leaching efficiency with a 2 and 3M HCl solutions was achieved with respect 1M HCl. With regard to the sample treated with a 3 M HCl solution (sample C4), the lowest signals of Ca-compounds were observed which were likely related to a very high leaching level of this element.

A raw material with a SiO_2/Al_2O_3 molar ratio close to one is recommended for the synthesis of A-type zeolite [6,8,11,14]. However, this material has been synthesized in some studies using a SiO_2/Al_2O_3 molar ratio ranging from 1.4 to 3.0 [6,28]. In this study, the formation of A-type zeolite was not promoted with SiO_2/Al_2O_3 molar ratio values higher than 1.88.

A comparison of the diffractograms illustrated in Figures 2 and 3 permits to determine that B4 and B8 samples showed the same pattern and the signal intensity in both cases was also similar. These signals correspond to P-type zeolite. The SiO_2/Al_2O_3 molar ratio for these samples was 3.67, which is very similar to values reported by other authors [7,10,12]. Additionally, some signals corresponding to kaolinite have also been identified. The presence of kaolinite is probably due to the fact that the calcination temperature could not transform all the kaolinite into metakaolinite.

In this work it was shown that the best leaching conditions for the formation of A-type zeolite were 1 M HCl solution and 8-hr synthesis time (A8 sample), while lixiviation with a 2M HCl solution and a minimum 4-hr synthesis time achieved the highest formation rate of P-type zeolite (B4 sample).

Figure 4 shows a SEM micrograph of the A8 particles sample. Cubic structures are observed, which corresponds with A-type zeolite morphology [28]. Particles with poorly defined shapes were also observed.

In Figure 5, some spherical particles can be observed in the B4 sample, corresponding to P-type zeolite [29] since this zeolite was identified in this sample by XRD.

CONCLUSIONS

In this research, the synthesis of A-, P- and NaP-CaP-type zeolites was successfully achieved by using paper sludge as raw materials. These zeolites were produced by applying sequentially the following processes to paper sludge: calcination, acid leaching and the alkaline hydrothermal process (AHP). The best AHP reaction time obtained in this study was lower than the average value reported in other similar works for

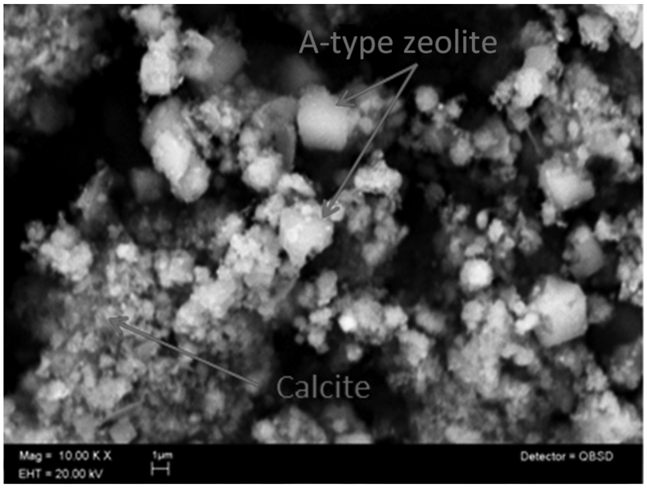

Figure 4. Micrograph of A8 sample particles: A-type zeolite and calcite.

Figure 5. Micrograph of B8 particles: P-type zeolite, NaP-CaP zeolite and calcite.

the A-zeolite synthesis and was similar to the average value reported for producing P-zeolite. This result and the use of wastes for the zeolites synthesis represent important economic advantages which could make the production of these adsorbents used in several industrial and environmental applications. This would also help to mitigate the contamination associated to the disposal of these wastes. The performance of tests necessary to evaluate the efficiency of these materials in the treatment of wastewaters and in the mitigation of atmospheric contamination through greenhouse gases is highly recommended.

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Biosolids Impact on Antioxidant Metabolism in Tall Fescue Under Drought Stress

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ABSTRACT: Biosolids are known to impact plant growth and drought tolerance. However, the underlying mechanisms are not well understood. This greenhouse study was designed to investigate whether biosolids improve antioxidant metabolism associated with drought tolerance in tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire]. All treatments in this experiment were applied with N availability equivalent to 75 mg N kg^{-1} soil: control (75 mg N kg^{-1} soil completely provided by NH_4NO_3 solution); biosolids at 0.5x agronomic N rate (37.5 mg N kg^{-1} soil provided by biosolids, and 37.5 mg N kg^{-1} soil provided by NH_4NO_3 solution); and biosolids at 1x agronomic N rate (75 mg N kg^{-1} soil completely provided by biosolids). Tall fescue was established and grown under well-watered or drought stress conditions. Biosolids improved tall fescue turfgrass quality and leaf relative water content (LRWC) under both water conditions and reduced leaf wilting rate under drought stress. Biosolids treatments also promoted leaf proline content and nitrate reductase activity under two moisture regimes and increased superoxide dismutase, catalase, ascorbate peroxidase, and peroxidase activity under drought stress. Biosolids increased tall fescue shoot and root biomass and improved root viability under drought stress. Tall fescue treated with biosolids showed better water status under drought stress. Biosolids application improved visual quality and leaf water status. The improvement of drought tolerance by biosolids treatments may be associated alteration in antioxidant enzyme activity.

INTRODUCTION

BIOSOLIDS are treated sewage sludges that have received an established treatment for a required level of pathogen control and have been treated or managed to reduce vector attraction to a specified level and they contain acceptable levels of pollutants in accordance with an issued permit [1]. It has been demonstrated that biosolids can not only ameliorate soil negative effects (e.g., loss of soil organic matter, loss of structure and permeability, and increased compaction) [2,3,4], but also enhance the biological and chemical properties of the soil and stimulate soil microbe growth and activity [3].

Drought is becoming a major threat to the sustainable development of areas lacking water. It imposes multiple stresses on plant, the resultant morphological, biochemical and molecular alterations negatively affected plant growth and productivity [5]. Among series consequences of drought effects on plant, nutrition restriction and water acquisition are the main targets under investigation [6].

Drought resistance of plants is associated with osmotic adjustment [7], antioxidant metabolism [8], N metabolism [9] as well as ideal phenotypes. Previous studies showed that the proline content increased during drought stress and enhanced drought tolerance in plants [10]. Antioxidant defense systems can protect plant cells from excess reactive oxygen species that are produced under environmental stress [8]. Nitrogen, N, metabolites are closely related to drought tolerance [9]. It has been proven that nitrate reductase activity (NRa) decreased in plants exposes to water limitation. NRa represents the ability of N assimilation of plants [11,12]. An ideal phenotype should not only maintain an adequate stomatal aperture for continued photosynthesis while tolerating the added expense of water loss, but also maintain a healthy, active root system for continued water uptake and supply to above ground tissues during water deficit [13]. Maintaining viable root systems that have high water conducting ability and high

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surface area for increased contact with soil is critically important for continued water uptake upon soil drying [14].

Previous studies indicated that biosolids contain biologically active substances (e.g., humic substances, amino acids, vitamins, hormones) [15,16] which can enable crops to withstand environmental stress, and enhance crop production [17]. Frankenberger *et al.* [18] noted that hormones found in the rhizosphere can influence plant growth and that the physiological effects of these materials cannot be replicated by an equivalent application of mineral nutrients. Recent studies indicated that biosolids can enhance the drought resistance ability of turfgrasses [19,20,8]. Zhang *et al.* [20] found that biologically active substances in biosolids provided plant growth regulators directly or stimulated the activity of microbes that supply substrates and hormones. Biosolids can enhance tall fescue quality and root mass, as well as up-regulation of N-rich defensive compounds under moisture stress.

Tall fescue [*Lolium arundinaceum* (Schreb.) S.J. Darbyshire], a cool-season turfgrass, is widely used for home lawns, recreational surfaces, and roadsides in temperate to semitropical areas. Little previous research has been reported on the impact of biologically active substances in biosolids on N metabolism and antioxidant metabolism associated with drought tolerance in tall fescue. Our objectives of this study were to investigate effects of biosolids application on drought tolerance associated with visual quality, N metabolism, antioxidant metabolism, as well as root characteristics under drought stress.

MATERIALS AND METHODS

Biosolids Characterization

The biosolids were collected at Jiuxianqiao Wastewater Plant of Beijing (Beijing, China). The characteristics of the biosolids were: water content 80.3%, total Kjeldahl N 60.5 mg, total P 19.3 mg g^{-1}, total K 7.1 mg g^{-1}, Ca 7.8 mg g^{-1}, Fe 3.5 mg g^{-1}, Mg 2.4 mg g^{-1}, Mn 0.052 mg g^{-1} and indole-3-acetic acid (IAA) 1.62 µg g^{-1}. The biosolids were stored in sealed plastic bags at 4°C before application.

Preliminary Actual Plant-Available Nitrogen of Biosolids Study

A preliminary experiment was conducted to determine the actual plant-available N provided by the fresh biosolids. The preliminary experiment consisted of five treatments: four inorganic N rates (0, 25, 50, and 75 mg N kg^{-1} soil added as NH_4NO_3 solution) and one treatment of biosolids applied at approximately 1.93g (dry weight) kg^{-1} soil. A randomized complete block design with five replications was used.

All treatments were added to pots (15-cm diam. by 11-cm depth) filled with 700g calcined clay (Profile Products, Chicago, IL USA) and seeded with tall fescue (Jagur 3) at 30 g m^{-2}, and treated with a full-strength N-free Hoagland's solution [21]. Before seeding and throughout the course of the experiment, pots were watered to 90% container capacity. We determined container capacity of calcined clay by filling five 15-cm-diampots with 700 g of calcined clay each, weighting them, saturating them with water, and then covering them with plastic film to prevent evaporation. The water was allowed to drain for 24 h, and the pots were then reweighed for determination of moisture content [20].

Tall fescue was clipped twice during the preliminary experiment which lasted for 8 weeks, and the clippings were dried at 65°C to analyze for total Kjeldahl N. Tall fescue N uptake (in mg N pot^{-1}) was determined by multiplying accumulative aboveground biomass pot^{-1} by tall fescue N concentration. Researchers calculated N provided by biosolids by means of a linear regression calibration equation: $Y = 0.402X + 16.24$ ($R^2 = 0.999$; $P < 0.001$), where Y represents N uptake (mg pot^{-1}) and X represents N applied (mg kg^{-1}). Thus plant-available N provided by biosolids was 36.24 mg kg^{-1}. Based on this data, a rate of 2.07 g biosolids (dry weight) kg^{-1} soil was used to supply 75 mg N kg^{-1} soil in the following experiment.

Tall Fescue Culture, Biosolids Application and Drought Stress Treatment

Tall fescue was cultivated in plastic pots (23-cm diam. by 15-cm depth) filled with 2.5 kg of calcined clay. All pots were seeded with tall fescue (Jagur 3) seeds at 30 g m^{-2} on 2 Aug 2012, full-strength N-free Hoagland solution was added to all pots to supply adequate P, K, and other macro- and micro-nutrients. Plants were grown in a greenhouse with photosynthetically active radiation at 400 µmol m^{-1} s^{-1} (at 1400 h) and 28/18°C (day/night). Pots were watered to 90% container capacity (CC) with tap water before seeding and were maintained at this moisture content until the moisture stress treatments were conducted.

The treatments were applied with nitrogen rate equivalent to 75 mg N kg^{-1} soil. The treatments included: (1) control (75 mg N kg^{-1} soil completely provided by NH_4NO_3 solution); (2) biosolids at 0.5× agronomic N rate (37.5 mg N kg^{-1} soil provided by biosolids, and 37.5 mg N kg^{-1} soil provided by NH_4NO_3 solution); (3) biosolids at 1× agronomic N rate (75 mg N kg^{-1} soil completely provided by biosolids). Five replicates per treatment were conducted. The biosolids and NH_4NO_3 solution (25 mg N kg^{-1} soil) were applied before planting. The rest of NH_4NO_3 solution was applied at 14 d (25 mg N kg^{-1} soil) and 28 d (25 mg N kg^{-1} soil) after planting.

After all NH_4NO_3 solution was applied, the tall fescue was grown for seven weeks. Turfgrasses were mowed to approximately 10 cm once a week and clippings were collected. Then tall fescue was subjected to two soil moisture regimes: well-watered and drought stress. The "well-watered" pots were maintained at 90% CC throughout the experiment. For drought stress treatment, the tall fescue was dried by allowing soil moisture to drop gradually to ≈20% of CC and then irrigated to 90% CC for one week. Samples of leaf tissue for biochemical constituents were taken from the top of the canopy on 17 October (normal moisture, 90% CC), 31 October (50% CC), 12 November (30% CC), 23 November (20% CC) and 4 December (90% CC, re-watered for 7 d). Samples were wrapped with aluminum foil, frozen with liquid N_2, and stored at –80°C until use. Root samples were collected on 23 November (20% CC) using soil auger and the calcined clay to backfill the holes. Soil moisture content was measured with a Theta Probe soil moisture sensor (ML2, Delta-T Devices, Cambridge, UK).

Turfgrass Quality, Leaf Wilting, and Leaf Relative Water Content (RWC)

Turfgrass quality ratings were based on a numerical scale from 1 to 9, 1 = brown, dead turfgrass, 6 = minimal acceptable turfgrass, 9 = ideal green, healthy turfgrass [22]. Leaf wilting was rated based on a visual scale of 0 to 100%, with 100% indicating complete, permanent wilting of the canopy. Leaf relative water content was determined according to [23].

Leaf and Root Nitrate Reductase (NRa) Activity

NRa was assayed in vivo by measuring the NO_2^- content in tissue that had been vacuum infiltrated with buffered NO_3^- solutions [24]. The leaves and roots from the non-stressed and stressed tall fescue were collected in the morning, between 8:30 and 11:00 solar time. Leaves and roots were cut into 5-mm sections. Approximately 300 mg of leaf punches and 300 mg of roots were placed in tubes containing 2 mL incubation medium consisting of 0.05 M Tris-HCl and 0.25 M KNO_3(pH 7.8). The tubes were sealed and kept in the dark at 30°C for 1 h. After that, 1 mL of 1% sulphanilamide1 M HCl and 1 mL of 0.01%N-1-napthyl-ethylenediamine hydrochloride were added in the incubation mixture. The optical density was measured at 540 nm with a spectrophotometer after 15 min to determine the nitrite content (UV-2802S, UNICO, Spain).

Leaf Proline Content

Frozen leaf samples (100 mg) were cut into 5mm sections, and then placed into tubes containing 4 mL of 3% 5-sulfosalicylic acid. The mixture was boiled in 100ºC water for 10 min. 2 mL of supernatant was mixed with 2mL acetic acid and 2mL acidic ninhydrin solution. The mixed liquid was then boiled at 100°C for 30 min, and then 3 mL methylbenzene was added to the mixed liquid. It was then vortexed 1 min and then rested for a moment. The supernatant was used for the analysis. The Proline content was determined spectrophotometrically at 520 nm with a spectrophotometer (UV-2802S, UNICO, Spain) [25].

Leaf Chlorophyll Content

Fresh leaf samples (50mg) were cut into 5mm sections, and then placed into tubes containing 10 mL of 95% ethanol. The mixture was placed in dark for 24 h. Chl content was measured at 645 nm and 669 nm with a spectrophotometer(UV-2802S, UNICO, Spain) [26] .

Leaf Antioxidant Enzyme Activity

Frozen leaf tissues (0.25g) were ground to a powder with a mortar and a pestle in liquid N and then the powder was homogenized with 4 mL cold extraction buffer [50 mM Na_2HPO_4/NaH_2PO_4, pH 7, 0.2 m Methylenediamine-tetraacetic acid (EDTA), and 1% polyvinylpyrrolidone]. The extracts were centrifuged at 12,000× gn for 20 min at 4°C. The supernatant was collected and used for the assay of antioxidant enzyme activity. Then leaf antioxidant enzyme activity for superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) was determined according to [8].

Root Biomass and Shoot Biomass

Root biomass per pot was determined at the end of the experiment by removing roots from the pots. The roots samples and collected clippings were dried at 65°C for 48 h and weighed. Shoot biomass collected during the experiment were dried at 65°C for 48 h and weighted.

Root Viability

About 0.4 g (fresh weight) of roots (whole roots with base and tips) was collected for the measurement of root viability using the modified 2, 3, 5-triphenyltetrazolium chloride (TTC) reduction technique [27]. The roots were incubated in the dark for 24 h in 0.6% TTC at 37°C, then rinsed with deionized water and ground in a mortar and pestle with ethyl acetate. The absorbance of the solution was measured at 490 nm with a spectrophotometer (UV-2802S, UNICO, and Spain). Five independent samples were determined for each treatment. Root viability was expressed by the reduction intensity of red tetrazoline.

Experiment Design and Data Statistical Analysis

Two experiments were conducted, one in which no soil moisture stress was imposed (well-watered regime) and one in which soil moisture was limiting, and one wilt and recovery cycle was imposed (moisture stress regime). Each experiment was arranged on a greenhouse bench as a completely randomized design with five replications. The data from each experiment were subjected to repeated-measures general linear model using a multivariate approach. In this model, the between-subject variable (biosolid treatments) main effect, the within-subject variable (sampling dates) main effect, and the interaction were analyzed. In addition, one-way analysis of variance was used for analysis of sampling date effect. Mean separations were performed using a protected Fisher's least significant difference at a 5% probability level [20].

RESULTS

Turfgrass Quality

Turfgrass quality declined under drought stress and recovered gradually to an acceptable level after re-watering. Biosolids at 1× agronomic N rate and 0.5× agronomic N rate improved the turfgrass quality relative to the control during drought and recovery periods. Biosolids at 1× agronomic N rate and 0.5× agronomic N rate increased the turfgrass quality rating by 10.6% and 6.1%, respectively, when compared to the control as measured on 23 November under drought stress (Table 1). Under well-watered condition, biosolids at 1× agronomic N rate and 0.5× agronomic N rate also increased turfgrass quality as measured on 23 November.

LRWC

LRWC was reduced during drought stress and recovered after re-watering in all pots. Biosolids at 1× agronomic N rate and 0.5× agronomic N rate increased LRWC by 14.3% and 12.7%, respectively, when compared to the control as measured on 23 November. After re-watering-biosolids at 1× agronomic N rate also increased LRWC (Table 1). No difference in LRWC was found between treatments under well-watered regimes.

Leaf Wilting

Leaf wilting was observed under drought stress. Biosolids treatments alleviated leaf wilting (Figure 1). Biosolids at 1× agronomic N rate and 0.5× agronomic N rate reduced leaf wilting by 23.3% and 7.9%, respectively, relative to the control as measured on 12 November (Figure 1).

Leaf NRa Activity

Drought stress reduced leaf NRa activity. Biosolids at 1× agronomic N rate and 0.5× agronomic N rate increased leaf NRa activity under drought stress regime. After re-watering, the grass treated with biosolids at 1× agronomic N rate and 0.5× agronomic N rate had greater NRa activity relative to the control (Table 2). Biosolids improved leaf NRa activity on two sampling dates (12 November and 4 December) under well-watered conditions.

Root NRa Activity

Drought stress reduced root NRa activity (Figure 2). Biosolids at 1× agronomic N rate increased root NRa activity by 19.3% when compared to the control as measured on 23 November.

Leaf Proline Content

Drought resulted in proline accumulation in tall

Table 1. Biosolids Impact on Turfgrass Quality and Leaf Relative Water Content of Tall Fescue for Two Moisture Regimes.

Moisture	Treatment	17 Oct.	31 Oct.	12 Nov.	23 Nov.	4 Dec.
				Turfgrass Quality —1-9; 9= ideal green; healthy turfgrass—		
Well-watered†	Control	8.2aA‡	7.8bB	7.4cC	7.1cD	7.2cD
	biosolids at 0.5x agronomic N rate	7.8bA	7.7cB	7.6bB	7.3bC	7.3bC
	biosolids at 1x agronomic N rate	8.1aA	8.0aB	7.8aC	7.5aD	7.4aE
Drought stress	Control	8.2aA	7.4bB	5.6cD	4.7cE	6.1cC
	biosolids at 0.5x agronomic N rate	7.8bA	7.5bB	5.8bD	4.9bE	6.2bC
	biosolids at 1x agronomic N rate	8.1aA	7.6aB	6.1aD	5.2aE	6.4aC
				Leaf relative water content %		
Well-watered†	Control	93.51aA	92.74aA	87.62aB	84.72bB	85.00aB
	biosolids at 0.5x agronomic N rate	91.48aA	90.36aAB	87.41aC	89.10aAB	86.54aC
	biosolids at 1x agronomic N rate	92.96aA	90.35aAB	88.48aBC	87.10abBC	86.90aC
Drought stress	Control	92.40aA	90.53aA	66.37aB	40.55bC	64.80bB
	biosolids at 0.5x agronomic N rate	92.53aA	88.74aA	68.51aB	45.69aC	68.22bB
	biosolids at 1x agronomic N rate	92.19aA	89.76aA	66.41aC	46.33aD	73.36aB

†Well-watered: well-watered regime (maintained at 90% container capacity throughout the course of the experiment).

Drought stress: drought and re-water regime (by withholding irrigation to allow soil moisture to drop gradually from 90% to ≈20% of container capacity (8% soil water content) and then re-watering).

‡Means with same lower case letters within column or uppercase letters within each row of each data set are not significantly different at $P < 0.05$.

fescue. Under drought stress, biosolids treatments increased proline content. Biosolids at 1× agronomic N rate and 0.5× agronomic N rate increased proline content by 33.6% and 9.5% relative to control on 23 November (Table 2).

Leaf Chlorophyll Content

Leaf chlorophyll content was reduced significantly after drought stress, and was enhanced after re-watering. There was no difference in leaf chlorophyll content between biosolids treatments under well-watered conditions. However, under drought stress, biosolids at 1× agronomic N rate and 0.5× agronomic N rate increased leaf chlorophyll content by 31.3% and 29.5%, respectively, on 12 November (Table 2).

Leaf Superoxide Dismutase Activity

Drought stress induced an increase in SOD activity as observed on 31 October, 12 November, and 23 November. Both biosolids treatments increased SOD activity, when compared to the control, on 31 October and 12 November under well-watered conditions. Under drought stress conditions, biosolids at 1× agronomic N rate and 0.5× agronomic N rate increased SOD activity relative to the control on 12 November

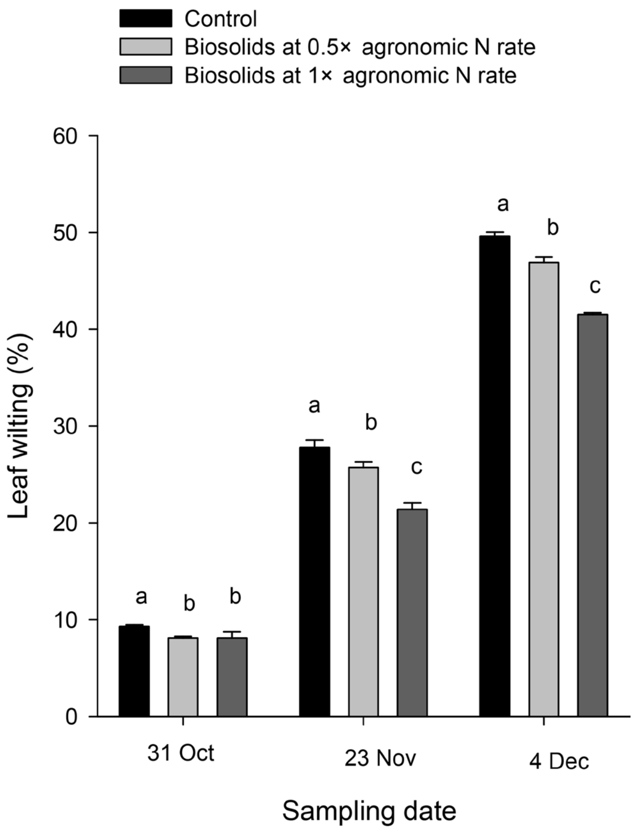

Figure 1. *Leaf wilting responses to biosolids application in tall fescue under drought stress on 31 October, 23 November, and 4 December marked with the same letter for each sampling date not significantly different at P < 0.05.*

Table 2. Biosolids Impact on Leaf Nitrate Reductase Activity, Leaf Proline Content, and Leaf Chlorophyll Content of Tall Fescue for Two Moisture Regimes.

Moisture	Treatment	17 Oct.	31 Oct.	12 Nov.	23 Nov.	4 Dec.
				Leaf NRa activity —μgNO_2-.$g^{-1}FW.h^{-1}$—		
Well-watered†	Control	33.09aA‡	28.76aB	28.09bB	27.50aB	27.22bB
	biosolids at 0.5x agronomic N rate	32.40aA	27.75aB	28.56bB	28.91aB	29.12abB
	biosolids at 1x agronomic N rate	34.99aA	29.75aC	31.89aB	29.22aC	29.44aC
Drought stress	Control	32.21bA	24.64aB	20.40bC	16.86bD	21.56bC
	biosolids at 0.5x agronomic N rate	33.64abA	26.56aB	23.60aC	21.87aC	23.09abC
	biosolids at 1x agronomic N rate	35.65aA	26.74aB	23.63aBC	23.18aC	25.45aBC
				Leaf proline content $\mu g.g^{-1}FW$		
Well-watered†	Control	8.46aB	9.11aB	7.33bB	7.53aB	14.26bA
	biosolids at 0.5x agronomic N rate	7.76aC	8.96aC	12.02aB	6.43aC	18.31aA
	biosolids at 1x agronomic N rate	6.08aB	7.07aB	5.06bB	5.78aB	19.04aA
Drought stress	Control	8.14aE	19.91bD	41.10bB	198.40cA	32.20cC
	biosolids at 0.5x agronomic N rate	8.83aE	19.55bD	44.56bB	217.30bA	35.04bC
	biosolids at 1x agronomic N rate	5.93bE	23.97aD	59.08aB	265.01aA	38.34aC
				Leaf chlorophyll content $mg.g^{-1}FW$		
Well-watered†	Control	2.95aA	2.35aB	2.29aB	2.16aB	2.16aB
	biosolids at 0.5x agronomic N rate	3.01aA	2.64aAB	2.42aB	2.27aB	2.26abB
	biosolids at 1x agronomic N rate	3.02aA	2.66aB	2.42aBC	2.20aC	2.56aB
Drought stress	Control	2.94aA	2.21aB	1.66bC	1.69aC	1.86bC
	biosolids at 0.5x agronomic N rate	3.04aA	2.41aB	2.15aBC	1.74aD	2.07aC
	biosolids at 1x agronomic N rate	2.96aA	2.43aB	2.18aBC	1.86aD	2.10aCD

†Well-watered: well-watered regime (maintained at 90% container capacity throughout the course of the experiment).

Drought stress: drought and re-water regime (by withholding irrigation to allow soil moisture to drop gradually from 90% to ≈20% of container capacity (8% soil water content) and then re-watering).

‡Means with same lower case letters within column or uppercase letters within each row of each data set are not significantly different at $P < 0.05$.

and 23 November Biosolids at 1× agronomic N rate and 0.5× agronomic N rate increased SOD activity by 32.8% and 15.3%, respectively, on 12 November, and by 52.9% and 23.4%, respectively, on 23 November relative to the control (Table 3).

Leaf Catalase Activity

Drought stress induced a decrease of CAT activity as observed on 31 October, 12 November and 23 November. No difference in CAT activity was observed between the treatments under well-watered conditions. Biosolids at 1× agronomic N rate and 0.5× agronomic N rate increased CAT activity relative to the control under drought stress (12 November and 23 November) and re-watering period (4 December).

Leaf Ascorbate Peroxidase Activity

Drought stress increased APX activity. Biosolids

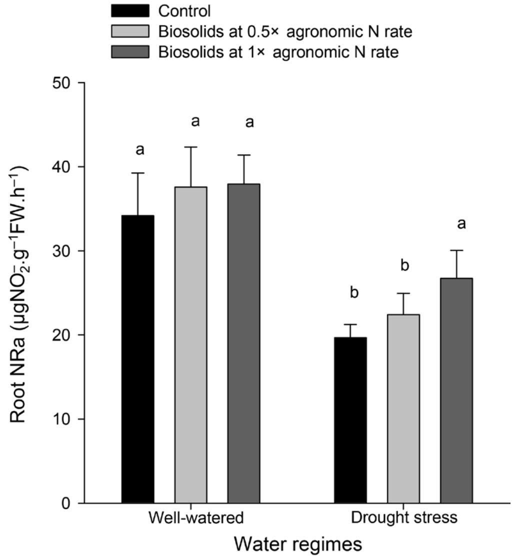

Figure 2. *Root nitrate reductase (NRa) activity responses to biosolids application in tall fescue subjected to two moisture regimes. Root samples were collected on 23 November. Bars marked with the same letter for each sampling dates are not significantly different at P < 0.05.*

at 1× agronomic N rate and 0.5× agronomic N rate increased APX activity under drought stress (12 November and 23 November). Biosolids at 1× agronomic N rate and 0.5× agronomic N rate increased APX activity by 15.6% and 11.5% relative to the control as measured on 23 November. After re-watering, a higher APX activity was observed in biosolids at 1× agronomic N rate relative to the control (Table 3). No difference in APX activity was found between the treatments under well-watered conditions.

Leaf Peroxidase Activity

Drought stress caused an increase in POD activity as measured from 31 October to 23 November Biosolids at 1× agronomic N rate and 0.5× agronomic N rate increased POD activity by 26.7% and 17.0%, respectively, when compared to the control under drought stress (23 November) (Table 3). Under well-watered conditions, biosolids at 1× agronomic N rate increased POD activity when compared to the control as measured on 23 November.

Table 3. Biosolids Impact on Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), and Peroxidase (POD) Activity of Tall Fescue for Two Moisture Regimes.

Moisture	Treatment	17 Oct.	31 Oct.	12 Nov.	23 Nov.	4 Dec.
				Leaf SOD activity U.mg^{-1} protein		
Well-watered†	Control	113.0aC‡	125.7bB	144.9bA	129.0aB	127.7aB
	biosolids at 0.5x agronomic N rate	111.1aC	138.6aB	156.6aA	137.8aB	131.7aB
	biosolids at 1x agronomic N rate	112.6aC	139.6aB	155.7aA	141.2aB	133.1aB
Drought stress	Control	115.5aC	141.6aB	179.5cA	174.2cA	144.5aB
	biosolids at 0.5x agronomic N rate	119.4aC	143.8aB	206.8bA	215.9bA	154.6aB
	biosolids at 1x agronomic N rate	113.9aC	146.0aB	224.5aA	231.8aA	147.3aB
				Leaf CAT activity U.min^{-1} protein.mg^{-1}		
Well-watered†	Control	64.2aA	64.3aA	57.8aAB	62.5aAB	56.8aB
	biosolids at 0.5x agronomic N rate	61.0aA	62.1aA	54.9aA	59.7aA	56.7aA
	biosolids at 1x agronomic N rate	65.1aA	58.1aB	56.5aB	58.5aB	58.5aB
Drought stress	Control	65.7aA	51.0aB	40.9bCD	37.9bD	45.6bC
	biosolids at 0.5x agronomic N rate	63.3aA	56.7aB	45.7abC	44.0aC	53.3aB
	biosolids at 1x agronomic N rate	61.7aA	56.5aAB	47.9aD	45.1aD	53.6aBC
				Leaf APX activity U.min^{-1}.mg^{-1} protein		
Well-watered†	Control	8.66aC	9.60aB	10.59aA	9.72aB	9.26aB
	biosolids at 0.5x agronomic N rate	8.71aC	9.86aB	10.77aA	9.86aB	9.13aAB
	biosolids at 1x agronomic N rate	8.57aC	10.12aB	10.33aA	9.89aB	9.17aAB
Drought stress	Control	8.51aD	10.34aC	17.49bB	19.36bA	17.65bB
	biosolids at 0.5x agronomic N rate	8.64aD	10.76aC	19.03aB	21.58aA	18.38abB
	biosolids at 1x agronomic N rate	8.47aD	10.95aC	19.95aB	22.38aA	19.05aB
				Leaf POD activity U.min^{-1} protein.mg^{-1}		
Well-watered†	Control	3.63aA	3.46aAB	3.51aAB	3.06bC	3.28aBC
	biosolids at 0.5x agronomic N rate	3.60aA	3.34aAB	3.46aAB	3.15abB	3.32aAB
	biosolids at 1x agronomic N rate	3.57aA	3.38aA	3.44aA	3.30aA	3.38aA
Drought stress	Control	3.66aB	6.50aB	8.98bB	10.54bA	10.83bA
	biosolids at 0.5x agronomic N rate	3.51aE	6.74aD	9.52aC	12.33aA	11.35bB
	biosolids at 1x agronomic N rate	3.62aE	6.99aD	9.71aC	13.36aA	12.65aB

†Well-watered: well-watered regime (maintained at 90% container capacity throughout the course of the experiment).

Drought stress: drought and re-water regime (by withholding irrigation to allow soil moisture to drop gradually from 90% to ≈20% of container capacity (8% soil water content) and then re-watering).

‡Means with same lower case letters within column or uppercase letters within each row of each data set are not significantly different at $P < 0.05$.

Shoot Biomass

Biosolids at 1× agronomic N rate and 0.5× agronomic N rate increased clipping weight by 17.3% and 7.4%, respectively, under well-watered conditions. Biosolids at 1× agronomic N rate also increased clipping production under drought stress regime (Figure 3).

Root Biomass

Biosolids at 1× agronomic N rate increased root biomass by 16.3% under well-watered condition, and by 39.2% under drought stress, respectively, when compared to the control (Figure 4).

Root Viability

Drought stress decreased root viability. Biosolids at 1× agronomic N rate and 0.5× agronomic N rate increased root viability by 25.0% when compared to the control under drought stress regime (Figure 5). No difference in root viability was found between treatments under well-watered condition (Figure 5).

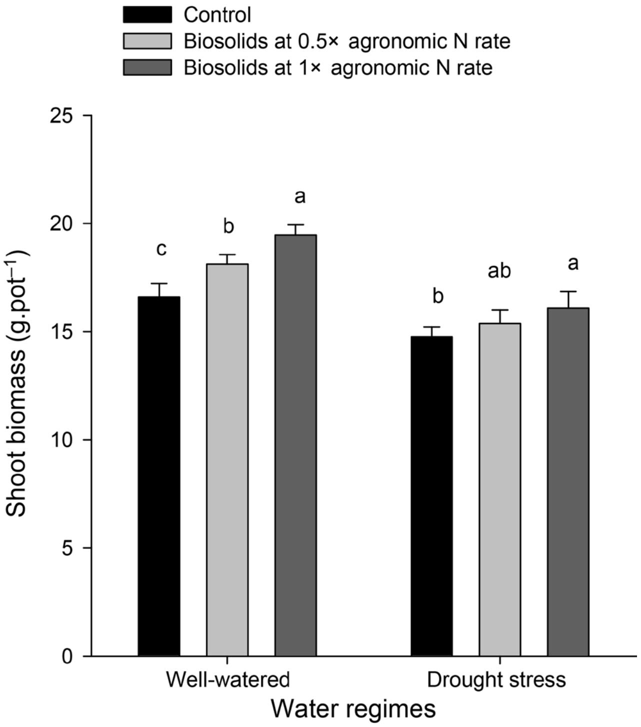

Figure 3. *Shoot biomass responses to biosolids application in tall fescue subjected to two moisture regimes. Bars marked with the same letter for each sampling dates are not significantly different at P < 0.05.*

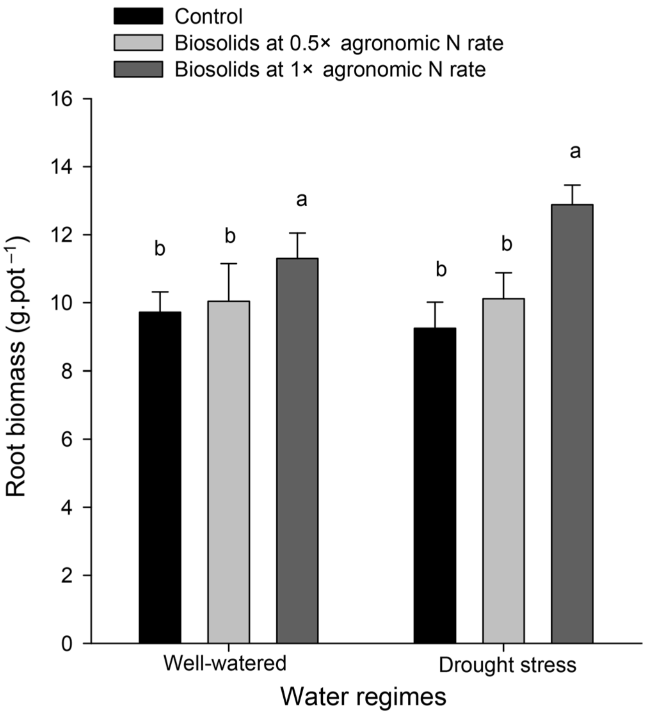

Figure 4. *Root biomass responses to biosolids application in tall fescue subjected to two moisture regimes. Root samples were collected at the end of trial. Bars marked with the same letter for each sampling dates are not significantly different at P < 0.05.*

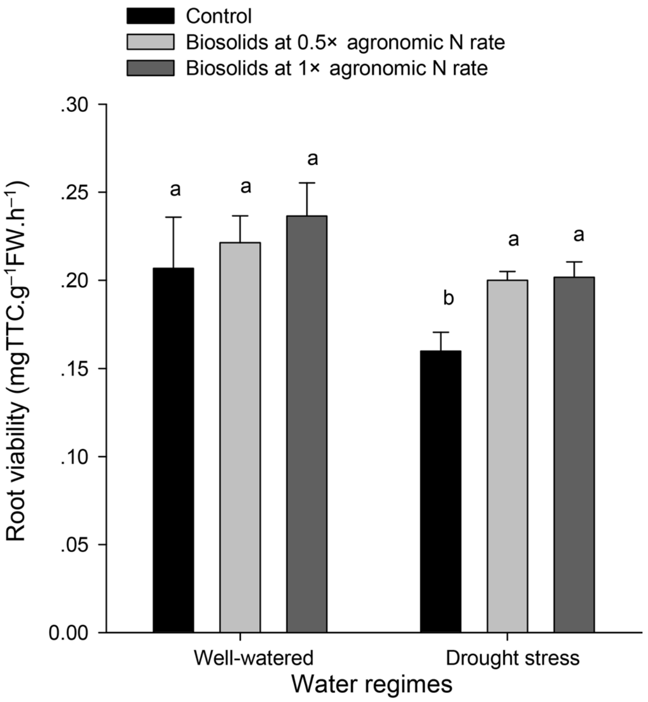

Figure 5. *Root viability responses to biosolids application in tall fescue subjected to two moisture regimes. Root samples were collected on 23 November (20% container capacity). Bars marked with the same letter for each sampling dates are not significantly different at P < 0.05.*

DISCUSSION

The results of this study indicated that biosolids improved turfgrass quality and LRWC under both moisture conditions, reduced leaf wilting under drought stress. This is consistent with previous reported results [19,20,8]. Our data suggests that biosolids improved water status of tall fescue during drought stress and promoted post-drought recovery. Beneficial effects of biosoids appear to result from factors other than mineral nutrition because all treatments in this study received an equivalent estimated amount of N and other macro- and micro-nutrients from chemical fertilizer and/or biosolids. In this study, IAA was isolated from biosolids; therefore, the biologically active substances in biosolids may play a positive role on tall fescue drought tolerance.

Our data showed that biosolids improved leaf and root NRa activity, leaf proline content and chlorophyll content. The positive effects of biosolids on leaf and root NRa activity suggest that biosolids can also improve N acquisition, particularly under drought stress conditions. This was similar with the result of [28] who found that the application of sewage sludge improved the N concentration of tall fescue. It has been reported that in vivo NRa activity can be modulated by many metabolic and physiology factors including carbohydrate availability [29]. The higher chlorophyll content of tall fescue treated with biosolids may promote photosynthesis. Proline, an amino acid, is a compatible solute involved in cell osmotic adjustment and protection of cell component during dehydration [23]. Our results indicated that biosolids increased leaf proline content under drought stress. This suggests biosolids may improve drought tolerance partially by enhancing osmoprotectants including proline. The results of this study also indicated that biosolids enhanced tall fescue leaf antioxidant enzyme activity under drought stress. This is consistent with previous reported results by Zhang *et al.* [19,20,8]. Drought induced excess accumulation of toxic reactive oxygen species (ROS) in cells, which may cause damage to cell components [30]. Various antioxidant metabolites and enzymes can scavenge toxic ROS and protect cells under stress. Our data showed tall fescue treated with biosolids had greater antioxidant enzyme (SOD, APX, CAT, and POD) activity under drought stress. This suggests that biosolids promote antioxidant defense against drought stress, and this may be caused by biologically active substances in biosolids. It has been reported that biosolids-fertilized crops had greater yields than crops fertilized with synthetic fertilizer where both were applied at rates estimated to provide adequate amounts of primary nutrients [31]. In our study, rooting characteristics were evaluated by measuring root biomass and viability. The results of this study showed that drought reduced root viability and decreased root biomass. However, application of biosolids improved root viability under drought stress. Moreover, the biosolids treatments increased root biomass under both soil moisture regimes. Since water uptake efficiency of roots is a desirable character of dehydration avoidance [32], viable roots have higher water conducting ability, and higher surface area for increased contact with soil is important for continued water uptake from drying soil [14]. Tall fescue treated with biosolids under drought maintained better water status (higher LRWC). The biosolids' induced improvement of drought tolerance may be partially related to the increased root growth and viability. Biosolids application has been shown to increase plant endogenous IAA content which enhances root growth [25]. Biosolids also improved soil biological and chemical properties, stimulated soil microbial activity [3].

In conclusion, biosolids promoted tall fescue visual quality under drought stress, and enhanced N metabolism under well-watered and drought stress conditions. Biosolids increased antioxidant enzyme activity under drought stress. Biosolids also increased root biomass and improved root viability. Our data suggests that biosolids promoted tall fescue antioxidant metabolism and N metabolism under drought stress, and enhanced tall fescue drought resistance. Biosolids application appears to be a practical approach to improve plant growth and visual quality in droughty areas.

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Table 5. Comparison of state-of-the-art matrix resins with VPSP/BMI copolymers.

Resin System	Core Temp. (DSC peak)	T_E	Char Yield, %
Epoxy (MY720)	235	250	30
Bismaleimide (H795)	282	>400	48
VPSP/Bismaleimide copolymer			
C379: H795 = 1.9	245	>400	50
C379: H795 = 1.4	285	>400	53

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