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Original Research Article

Biostimulating Effects of Wastewater Sludges on Stressed Soils

F. Olcay Topaç Şağban*, Efsun Dindar and Hüseyin S. Başkaya
Uludag University, Faculty of Engineering, Department of Environmental Engineering, 16059, Görükle, Bursa, TURKEY

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ABSTRACT

Soil pollution is accepted as a pressing problem in many parts of the world resulting in efforts to develop a variety of soil remediation technologies. Properly processed wastewater sludge which contains significant amounts of nitrogen, phosphorus, organic matter and other trace elements is accepted as a promising nutrient source for microbes in soils contaminated with various contaminants. The objective of the present study was to determine the potential use of municipal wastewater sludge as a biostimulating agent in soils which has been irrigated with a polluted river water for long time and evaluate the soil enzyme activities. The results of 45 days incubation period indicated a significant improvement (1.5 to 5 fold in comparison to unamended polluted soil) in urease, alkaline phosphatase, dehydrogenase and β-glucosidase activities in sludge amended soils. Maximum values were observed in soils amended with 200 t/ha sludge. Although, the sludge dose of 100 t/ha increased the enzyme activity levels of polluted soil significantly, it seemed to be inadequate to increase activity levels of polluted soil to reference soil levels. Consequently, municipal sludge amendment with dose of 200 t/ha appeared to enhance the nutrient status of soil system, providing the removal of the anthropogenic stress caused by the irrigation from a polluted river.

Key Words: Biostimulation, enzyme activities, remediation, soil pollution, wastewater sludge

Arıtma Çamurlarının Stres Altındaki Topraklarda Biyostimülasyon Etkileri

ÖZET

Toprak kirliliği dünyanın pek çok yerinde önemli bir problem olarak kabul edilmekte ve çeşitli toprak temizleme teknolojilerinin geliştirilmesi için yoğun çaba harcanmasına neden olmaktadır. Önemli miktarda azot, fosfor, organik madde ile diğer iz elementlerini içeren ve uygun şekilde işlenmiş olan arıtma çamurları, çeşitli kirleticilerle kirlenmiş olan topraklardaki mikroorganizmalar için önemli bir bitki besin elementi kaynağıdır. Bu çalışmanın amacı kentsel bir arıtma çamurunun, uzun yıllardır kirli bir nehirden sulanan topraklarda biyostimülasyon maddesi olarak kullanılabilme potansiyelini araştırmak ve topraktaki enzim aktivitelerini değerlendirmektir. 45 günlük inkübasyon çalışması sonuçları çamur uygulanmış topraktaki üreaz, alkali fosfataz, dehidrogenaz ve β-glukosidaz aktivitelerinin önemli derecede geliştiğini (uygulama yapılmamış toprağa kıyasla 1,5 ila 5 kat arttığını) göstermiştir. En yüksek değerler 200 ton/ha oranında çamur uygulanan topraklarda gözlenmiştir. 100 ton/ha oranındaki çamur uygulaması kirlenmiş topraktaki enzim aktivitesi seviyelerini önemli derecede arttırmakla birlikte, bu seviyeleri, bölgedeki kirlenmemiş referans toprak seviyelerine getirmeye yeterli olmamıştır. Sonuç olarak, 200 ton/ha oranında yapılan kentsel arıtma çamuru uygulamasının toprak sisteminin bitki besin elementi durumunu geliştirdiği ve kirlenmiş nehirden yapılan sulamanın neden olduğu antropojenik baskıyı ortadan kaldırdığı izlenimi edinilmiştir.

Anahtar Kelimeler: Arıtma çamuru, biyostimülasyon, enzim aktiviteleri, ıslah, toprak kirliliği

INTRODUCTION

Solid waste disposal, irrigation with poor quality water, motor vehicle exhaust emissions and over use of fertilizers and pesticides are the major sources of soil pollution, and soil microbes and their associated biochemical processes are often depressed by the several direct and/or indirect effects of the pollutants. In addition to possible effects on human health (Tang et al. 2005, Juozulynas et al. 2008, Kah et al. 2012), elevated levels of soil pollutants can negatively affect plant vigor, animal health and agricultural production yields (Bruce et al. 2003, Wang et al. 2003, Wang et al. 2007, Kalavrouziotis et al. 2012). Soil pollution is accepted as a pressing problem in many parts of the world resulting in efforts to develop a variety of soil remediation technologies. Bioremediation, one of the environmental clean-up methodologies is defined as use of biological

* Corresponding author: olcaytopac@uludag.edu.tr

processes to degrade, break down, transform, and/or essentially remove contaminants or impairments of quality from soil. It offers the possibility to destroy or render harmless various contaminants using natural biological activity. This approach uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site.

Bioremediation of soil contaminants can be accomplished by two methods, bioaugmentation and/or biostimulation. The process of biostimulation introduces additional nutrients in the form of organic and/or inorganic fertilizers into a contaminated system, which increases the population of the indigenous microorganisms (Pankrantz 2001). The indigenous microorganisms may or may not primarily target the contaminant as a food source. However, they are assumed to degrade more quickly in comparison to natural attenuation due to the increased numbers of microorganisms caused by increased levels of nutrients. Various nutrient sources such as inorganic fertilizer, urea, crop residues, sawdust, compost, manure, and wastewater sludges have been used in biostimulation (Williams 1999, Molina-Barahona 2004). Properly processed wastewater sludge which contains significant amounts of nitrogen, phosphorus, sulphur, organic matter and other trace elements, represents a good source of nutrients for plant growth and a good soil conditioner to improve physical and chemical properties of soil (Epstein 2002; Davis 1989). These properties made sludge a promising nutrient source for microbes in soils contaminated with various contaminants such as petroleum products, PAHs, PCBs, pesticides, etc. (Sanchez et al. 2004, Contreras-Ramos et al. 2009, Topaç-Şağban et al. 2010, Topaç-Şağban 2011).

Soil as a biological system contains numerous enzymes derived from the microbial community, soil animals, plant residuals and root systems. Soil enzymes are accepted as possible integrative measure of soil quality to reflect existing status of soils since they are involved in microbial cycling of nitrogen, phosphorus and carbon. The rate of enzyme production and the activity-stability of enzymes are controlled by environmental conditions and ecological interactions (Acosta-Martinez and Tabatabai 2000). Therefore, enzyme activities in soil are accepted as bio indicators of anthropogenic stress caused by several soil pollutants and simple risk assessment tools in monitoring of contaminated sites.

The objective of the present study was to determine the potential use of tested wastewater sludge as a biostimulating agent in soils which has been irrigated with polluted river (Nilüfer-Ayvali Stream) water for more than 20 years and evaluate soil enzyme activities as potential indicators that provide an integrative biological assessment of contaminated soils amended with wastewater sludge.

MATERIALS AND METHODS

Materials

Soil samples were collected from the top 20 cm of an agricultural field near highly polluted Ayvalı/Nilüfer stream in Bursa-Nilüfer-Balabancık region. This stream is an important water source for the industrial and metropolitan city of Bursa, Turkey. Due to the lack of comprehensive wastewater management, effluent from households and industry have significantly polluted the stream. It was stated in many studies that Nilüfer-Ayvali Stream is heavily polluted owing to untreated wastewater discharges especially from textile and metal industries (Kaynak. 2002). As the adjacent arable fields have been irrigated with this stream water for many years, the surrounding soil is also at risk of being polluted. A soil sample from the same area that has not been irrigated with the polluted stream was also collected in order to determine the background levels of soil enzyme activities.

Dewatered sludge samples were collected from Yenice municipal treatment plant. General properties of soil and sludge samples included in this study are given in Table 1.

Table 1. General characteristics of soil and sludge samples.

Parameters[a]	Stressed Soil (irrigated)	Control Soil (nonirrigated)	Wastewater Sludge
Sand, %	56	54	-
Silt, %	19	17	-
Clay,%	25	29	-
Texture	Sandy clay loam	Sandy clay loam	-
pH (1:5, solid:water)	7.34	7.56	7.09
$EC_{25}o_C$ (1:5, solid:water), μS 25^oC	223	188	4260
Total-N, mg.kg^{-1}	866	785	63500
Ammonium-N, mg.kg^{-1}	26.6	44.3	2007
Nitrate-N, mg.kg^{-1}	19.6	12.6	349
Total-P, mg.kg^{-1}	720	548	3208
Available-P, mg.kg^{-1}	25.2	34.1	124
Easily oxidizable organic-C, % (w/w)	1.26	1.38	12.2

[a]on dry weight base

Incubation study

The 100 g soil portions were placed into plastic pots and air dried sludge samples were added to soil pots with doses of 0, 100 and 200 tons/ha. Control treatments without sludge were also included. Sludge amended and control pots were incubated under controlled conditions in the dark at 28◦C for 15, 30 and 45 days. The experiment was planned with a completely randomised design and each treatment was performed in triplicate to give a total of 27 experimental units at the start of the incubation. The soil moisture content was maintained at 70% of field capacity by the addition of distilled water as required. At each sampling time three sets of soil pots were removed and urease, alkaline phosphatase, dehydrogenase and β-glucosidase activities were determined.

Analysis of enzyme activities

The potential enzymatic activity of urease, dehydrogenase, alkaline phosphatase and β-glucosidase were determined according to the methods described by Tabatabai (1982).

The assay for urease activity was based on the determination of NH_4^+ released by urease when soil was incubated with THAM (Tris hydroxymethyl aminomethane) buffer (pH=9), urea solution (0.02 M) and toluene at 37 ^oC for 2 hours. The formation of ammonium was determined by steam distillation and results were expressed as μg NH_4^+-N g^{-1} h^{-1}.

In order to determine the dehydrogenase activity, triphenyl tetrazolium chloride solution (3 %) was added to soil and the suspension was incubated at 37^oC for 24 hours. The formation of TPF (triphenyl formazan) was determined spectrophotometrically at 485 nm and the results were expressed as μg TPF g^{-1} h^{-1}.

Alkaline phosphatase activities were performed by addition of modified universal buffer (pH=11), toluene and p-nitrophenyl phosphate solution (0.025 M) to soil and incubation of the soil suspension at 37^oC for 1 hour. Released (PNP) p-nitrophenol was determined spectrophotometrically at 410 nm and the results were expressed as μg PNP g^{-1} h^{-1}.

β-glucosidase activity test was based on colorimetric determination of p-nitrophenol released by the enzyme when soil was incubated with buffered PNG (p-nitrophenyl-β-D-glucoside) solution as substrate and toluene. Released PNP (p-nitrophenol) was determined spectrophotometrically at 410 nm and the results were expressed as μg PNP g^{-1} h^{-1}.

Statistical Analysis

Statistical analysis was performed by Analysis of Variance (ANOVA). Tukey's HSD multiple comparison test was used to evaluate significant differences between means. All statistical calculations were performed using STATISTICA 6.0 software.

RESULTS AND DISCUSSION

Considerable variations in all enzyme activities were observed for the different sludge doses at different incubation times. The results of the 2-way ANOVA test revealed that urease, β-glycosidase, dehydrogenase and alkaline phosphatase activities were significantly dependent on sludge dose and incubation time (Table2, $p<0.001$).

Table 2. Results of 2-way ANOVA test.

Sources of Variation	*df*	*MS*	*F*	*p*
Dependent variable: Urease activity				
Sludge dose (SD)	2	69609	208.7	<0.001
Incubation time (IT)	2	8622	25.85	<0.001
SDxIT	4	984.0	2.950	<0.05
Error	18	333.6		
Dependent variable: :β-Glycosidase activity				
Sludge dose (SD)	2	25435	42.46	<0.001
Incubation time (IT)	2	3080	5.142	<0.05
SDxIT	4	1595	2.662	n.s.[a]
Error	18	599.1		
Dependent variable: Alkaline Phosphatase activity				
Sludge dose (SD)	2	2279E3	375.5	<0.001
Incubation time (IT)	2	99654	16.42	<0.001
SDxIT	4	65483	10.79	<0.001
Error	18	6070		
Dependent variable: Dehydrogenase activity				
Sludge dose (SD)	2	1616E2	108.8	<0.001
Incubation time (IT)	2	32286	21.72	<0.001
SDxIT	4	10896	7.330	<0.01
Error	18	1487		

[a]n.s.: not significant

Figure 1 shows the effects of sludge amendments on urease activity of soils. According to the results of incubation, application of sludge inclined to increase urease activity at all incubation times. A1 amendment increased the urease activity approximately 1.9 fold in comparison to PS at 15th day of incubation. Thereafter activity values decreased and generally approximated to PS values at the end of the incubation. Tukey's HSD test ($p<0.05$) indicated that no significant difference was observed between the activity levels of A1 and PS at the end of incubation.

It is clear from Figure 1 that sludge dose of 100t/ha was not adequate to increase urease activities in polluted soil to reference levels (100.25-110.44µg NH_4^+-N/g soil.h). The recorded activity in A2 amendment was much more higher ($p<0.05$) than the value recorded in PS (3.2 fold), indicating the short-term stimulation impact of higher dose of sludge on urease activity. Although the activity level indicated a slight decreasing trend throughout the incubation, it was still significantly higher than the PS values at the end of incubation. Urease activity levels in A2 amendments were also apparently higher than the unpolluted reference soil at all incubation periods. Stimulated urease activity in A2 pots reflects the enhanced hydrolyzing capacity of C-N bonds of amide and urea. The municipal wastewater sludge sample appeared to have high levels of substrates capable of activating the enzyme synthesis (Benitez et al. 2005, Bastida et al. 2006).The effect of organic compounds in the studied wastewater sludge on soil urease activity is probably a combined effect of a higher degree of stabilization of enzymes to humic substances and an increase in microbial biomass with increased soil carbon concentration (Martens et al. 1992).

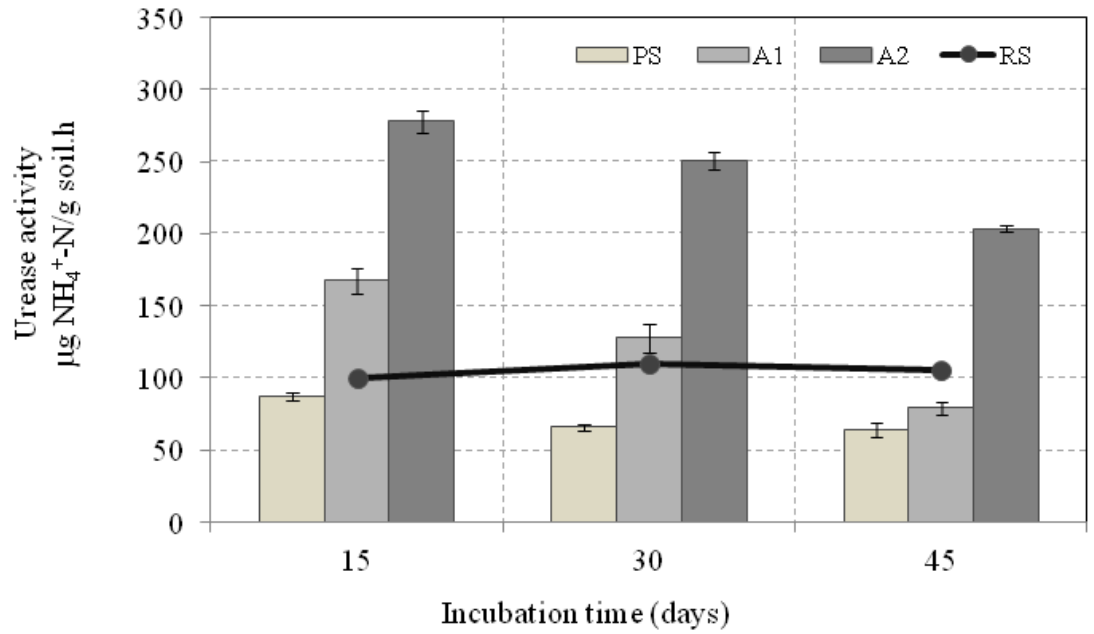

Figure 1. The variation of urease activities in soils amended with wastewater sludge (PS: polluted soil, A1: sludge amended polluted soil-100t/ha, A2: sludge amended polluted soil-200 t/ha, RS: reference soil).

Figure 2 depicts the effects of sludge amendments on β-glucosidase activity of polluted soil. The results of incubation indicated that the recorded β-glucosidase activity in A1 and PS was similar at 15th day of incubation. Thereafter activity levels significantly ($p<0.05$) increased (1.3 fold) and approximated to reference levels at day 30. However, this stimulation effect was not permanent. β-glucosidase activity in A1 decreased again to PS levels at the end of incubation. In case of A2 amendment, significant increases in β-glucosidase activity occurred at all incubation periods in agreement with other reports on the enhancement of hydrolytic enzymes by organic amendments (Jordan et al. 1995, Tejada et al. 2006). A2 amendment increased the β-glucosidase activity 1.5-1.8 fold and 1.2-1.6 fold in comparison to the levels in PS and RS. Glucosidases are widely distributed in nature and their hydrolysis products as low molecular weight sugars are important source of energy for soil microorganisms (Bandick and Dick 1999). The results of this incubation study indicated that sludge amendment with dose of 200 t/ha seemed to encourage the microbial communities in long term stressed soils.

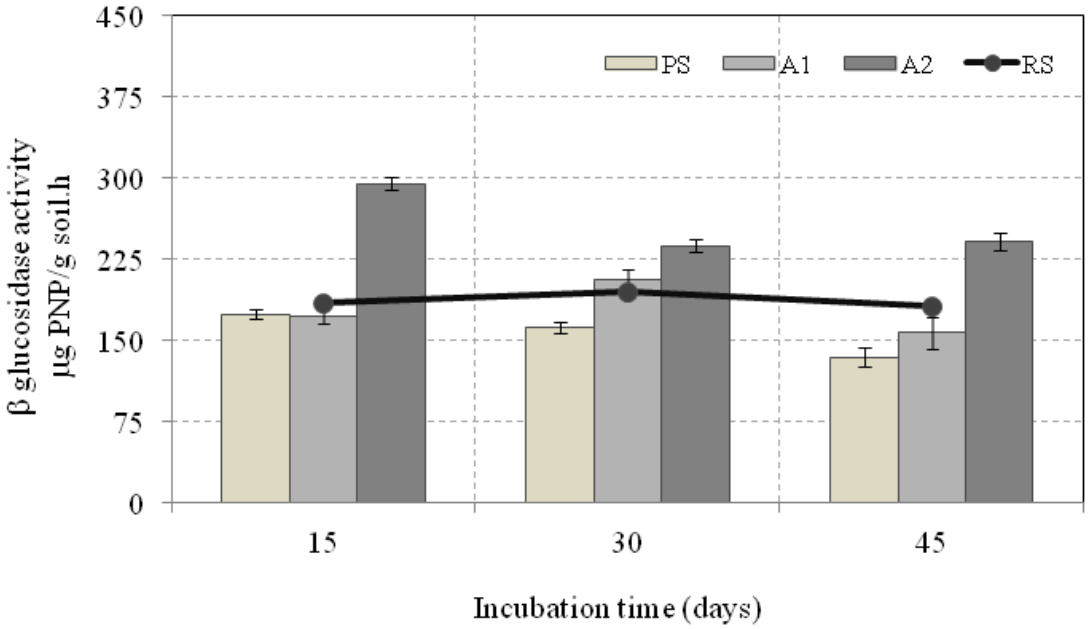

Figure 2. The variation of β-glucosidase activities in soils amended with wastewater sludge (PS: polluted soil, A1: sludge amended polluted soil-100t/ha, A2: sludge amended polluted soil-200 t/ha, RS: reference soil).

The effects of municipal sludge amendment on alkaline phosphatase activity of soils were presented in Figure 3. Tukey's HSD test ($p < 0.05$) indicated that no significant differences in alkaline phosphatase activity occurred between PS and A1 amendments after the incubation period of 15 days. The activity level in A1 amendment increased 4.4 fold in comparison to PS at day 30. Although the activity level in A1 apparently decreased at the end of the incubation, it still remained higher than the levels in PS and RS at the end of incubation. Sludge dose of 100t/ha appeared to be adequate to enhance alkaline phosphatase activity levels of pollued soil to reference soil levels.

The A2 amendment significantly increased (6.8 to 7.3 fold) the alkaline phosphatase activities at all incubation periods. Phosphatases are considered key enzymes in the phosphorus cycling in soil (Dick and Tabatabai 1993). Variations in phosphatase activity, apart from indicating changes in the quantity and quality of soil phosphorated substrates (Rao and Tarafdar 1992), are also good indicators of soil biological status. Alkaline phosphomonoesterase activity has not been detected in plants (Juma and Tabatabai 1988) and for this reason microbial cells supposedly synthesise most of the soil alkaline phosphomonoesterases (Tabatabai 1980). Both soil bacteria and soil microorganisms other than arbuscular mycorrhizal fungi are thought to contribute to the measured soil alkaline phosphomonoesterase activity (Joner and Jakobsen 1995). Thereby, it may be concluded that sludge application with dose of 200 t/ha provided a noticeable increment in microbial activity of the studied soil.

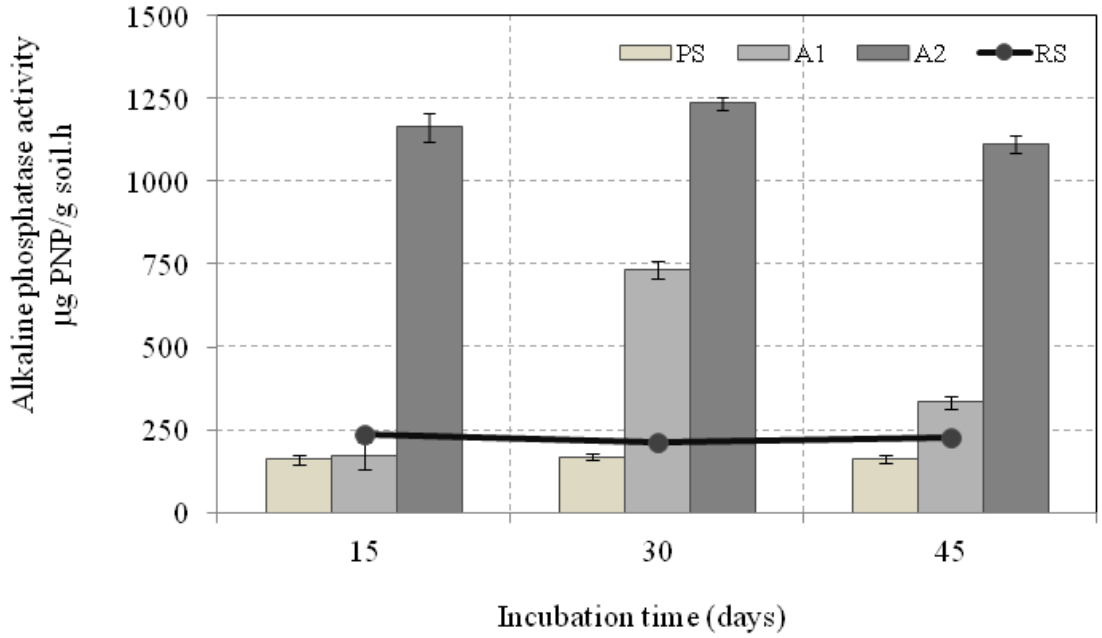

Figure 3. The variation of alkaline phospatase activities in soils amended with wastewater sludge (PS: polluted soil, A1: sludge amended polluted soil-100t/ha, A2: sludge amended polluted soil-200 t/ha, RS: reference soil).

As indicated in Figure 4, A1 amendment maintained higher levels of dehydrogenase activity in the soil in comparison to the values measured in PS during the study period. Although the activity levels in A1 amendment showed a decreasing trend till the end of incubation, it was still above the activity levels of PS at day 45. Dehydrogenase activities measured in A1 pots were similar to those in RS pots at days 15 and 30, whereas it was significantly ($p<0.05$) lower than the value of reference soil at day 45. Application of sludge with dose of 100t/ha initially stimulated the dehydrogenase activities, however, they were not maintained at the same high level over time.

The addition of A2 increased the dehydrogenase activity by an average of 5 fold when compared with the unamended polluted soil during the 45 days-incubation study. The peak dehydrogenase activity value was observed after 15 days of incubation which means a 7 fold increase in comparison to PS. Thereafter the activity value decreased to a steady level which was still higher than that of unamended polluted soil. No significant difference appeared between the dehydrogenase activities of A2 at day 30 and 45. When the enhanced dehydrogenase activity levels in A2 pots were compared to those observed in reference soil, the difference was statistically different ($p<0.05$) at all incubation periods. Sludge amendment with dose of 200t/ha appeared to increase dehydrogenase activity of polluted soil over natural background levels. The measured values in A2 pots were 1.5 to 2.5 fold higher than those in RS pots.

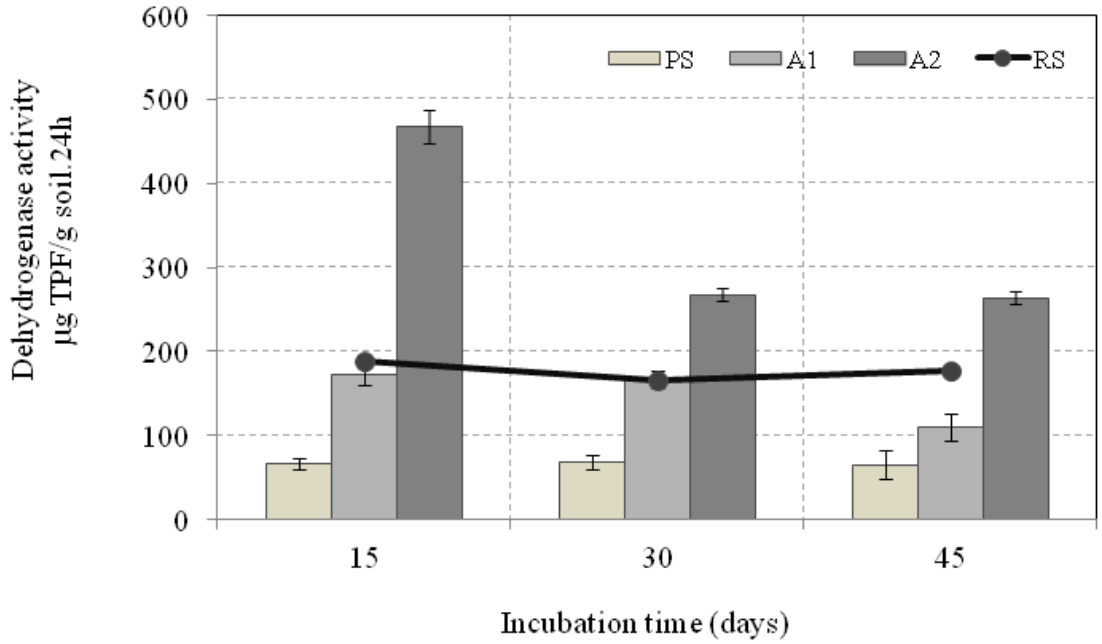

Figure 4. The variation of dehydrogenase activities in soils amended with wastewater sludge (PS: polluted soil, A1: sludge amended polluted soil-100t/ha, A2: sludge amended polluted soil-200 t/ha, RS: reference soil).

Dehydrogenase activity is used by researchers for evaluating the activity of soil microorganisms because dehydrogenase is not active independent of the parent microbial cell as an extra-cellular enzyme in soil. Therefore, the measurement of dehydrogenase activity is a good overall indicator of soil microbial activity. Consequently, the enhanced dehydrogenase activity levels observed in this study may be accepted as an indicator of enhanced microbial metabolic activity due to the incorporation of sludge originated compounds capable of activating the soil's biomass (Pascual et al. 1998, Franco-Otero et al. 2012). Addition of microbial cells present in wastewater sludge may also contributed to this enhancement.

CONCLUSION

A general conclusion which might be reached regarding the results of incubation experiments is that a significant improvement in urease, alkaline phosphatase, dehydrogenase and β-glucosidase activities of soils was occurred in wastewater sludge amended soils. In general 1.5 to 5 fold increases were measured in activity levels. Maximum values were observed in soils amended with 200 ton/ha sludge. Although, the sludge dose of 100t/ha increased the enzyme activity levels of polluted soil significantly, it seemed to be inadequate to increase activity levels of polluted soil to reference soil levels.

Municipal sludge amendment appeared to enhance the nutrient status of soil system, providing the removal of the anthropogenic stress caused by the irrigation from the polluted stream. Consequently, wastewater sludges from municipal sources may be used as an effective and economic biostimulating agent for agricultural lands polluted with several contaminants. The results also demonstrated that, among enzyme activities observed in this study, alkaline phosphatase and dehydrogenase activity were likely to show quicker and more apparent response to sludge amendments. These activities may be evaluated as an easy, rapid, and low cost procedure to monitor the soil health of soils amended with biostimulating agents.

REFERENCES

Acosta-Martinez V and Tabatabai MA (2000). Enzyme activities in a limed agricultural soil. Biology and Fertility of Soils. 31: 85-91.

Bandick AK and Dick RP (1999). Field management effect on soil enzyme activities, Soil Biology and Biochemistry. 31: 1471-1479.

Bastida F, Moreno JL, Hernández T and García C (2006). Microbiological activity in a soil 15 years after its devegetation. Soil Biology and Biochemistry. 38: 2503 - 2507.

Benitez E, Sainz H and Nogales R (2005). Hydrolytic enzyme activities of extracted humic substances during the vermicomposting of a lignocellulosic olive waste. Bioresource Technology. 96: 785-790.

Bruce FM, Fiona AN, Nnanna CU, Brian JC, James AH and Tom CJ (2003). Zinc contamination decreases the bacterial diversity of agricultural soil. FEMS Microbiology Ecology. 43: 13-19.

Contreras-Ramos SM, Alvarez-Bernal D and Dendooven L (2009). Characteristics of earthworms (*Eisenia fetida*) in PAHs contaminated soil amended with sewage sludge or vermicompost. Applied Soil Ecology. 41: 269-276.

Davis RD (1989). Agricultural utilization of sewage sludge: a review. Water and Environmental Journal. 3: 351-355.

Dick WA and Tabatabai MA (1993). Significance and potential uses of soil enzymes. In: Soil Microbial Ecology: Application in Agricultural and Environmental Management (Ed.: F.B. Metting). Marcel Dekker, New York, pp. 95-125.

Epstein E (2002). Land Application of Sewage Sludge and Biosolids. CRC Press, 216p.

Franco-Otero GV, Soler-Rovira P, Hernandez D, Lopez-de S, Esther G and Plaza C (2012). Short-term effects of organic municipal wastes on wheat yield, microbial biomass, microbial activity, and chemical properties of soil. Biology and Fertility of Soils. 48: 205-216.

Joner EJ and Jakobsen I (1995). Growth and extracellular phosphatase activity of arbuscular mycorrhizal hyphae as influenced by soil organic matter. Soil Biology and Biochemistry. 27: 1153-1159.

Jordan D, Kremer RJ, Bergfield WA, Kim KY and Cacnio VN (1995). Evaluation of microbial methods as potential indicators of soil quality in historical agricultural fields. Biology and Fertility of Soils. 19: 297-302.

Juma NG and Tabatabai MA (1988). Hydrolysis of organic phosphates by corn and soybean roots. Plant and Soil. 107: 31-38.

Juozulynas A, Jurgelėnas A, Butkienė B, Greičiūtė K and Savičiūtė R (2008). Implications of soil pollution with heavy metals for public health. Geologija. 50: 75-79.

Kah M., Levy L and Brown C (2012). Potential for effects of land contamination on human health. 1. The case of cadmium. Journal of Toxicology and Environmental Health Part B:Critical Review. 15: 348-363.

Kalavrouziotis IK, Koukoulakis PH, Ntzala G and Papadopoulos AH (2012). Proposed indices for assessing soil pollution under the application of sludge. Water, Air & Soil Pollution. 223: 5189-5196.

Kaynak AG (2002). Treatment of wastewaters originated from Bursa city centre and the effects on Nilüfer Stream, M. Sc. thesis, Uludag University, Bursa, Turkey.

Martens DA, Johanson JB and Jr. Frankenberger WT (1992). Production and persistence of soil enzymes with repeated addition of organic residues. Soil Science. 153: 53-61.

Molina-Barahona L, Rodriguez-Vazguez R, Hernandez-Velasco M, Vega-Jarguin C, Zapata-Perez O, Mendoza-Cantu A and Albores A (2004). Diesel removal from contaminated soils by biostimulation and supplementation with crop residues. Applied Soil Ecology. 27: 165-175.

Pankrantz TM (2001). Environmental Engineering Dictionary and Directory, CRC Press, Boca Raton, FL.

Pascual JA, Hernandez T, Garcia C and Ayuso M (1998). Enzymatic activities in an arid soil amended with urban organic wastes: Laboratory experiment. Bioresource Technology. 64: 131-138.

Rao AV and Tarafdar JC (1992). Seasonal changes in available phosphorus and different enzyme activities in arid soils. Annals of Arid Zones. 31: 185-189.

Sanchez ME, Estrada IB, Martinez O, Martin-Villacorta J, Aller A and Moran A (2004). Influence of the application of sewage sludge on the degradation of pesticides in the soil. Chemosphere. 57: 673-679.

Tabatabai MA (1982). Soil enzymes. In: Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties (Eds.: A.L. Page, R.H. Miller, D.R. Keeney). ASA and SSSA, Madison, WI, pp. 903-943.

Tang L, Tang XY, Zhu YG, Zheng MH and Miao QL (2005). Contamination of polycyclic aromatic hydrocarbons (PAHs) in urban soils in Beijing, China. Environment International. 31: 822-828.

Tejada M, Hernandez MT and Garcia C (2006) Application of two organic amendments on soil restoration: effects on the soil biological properties. Journal of Environmental Quality. 35: 1010-1017.

Topac FO, Dindar E, Uçaroğlu S and Başkaya HS (2010). Biostimulation of azo dye-contaminated soils by food industry sludge. Soil and Sediment Contamination An International Journal. 19: 436-454.

Topac-Sagban FO **(**2011). Impacts of wastewater sludge amendments in restoring nitrogen cycle in *p*-nitrophenol contaminated soil. Journal of Environmental Science. 23: 616-623.

Wang QR, Cui YS, Liu XM, Dong YT and Christie P (2003). Soil contamination and plant uptake of heavy metals at polluted sites in China. Journal of Environmental Scıence and Health Part A-Toxıc/hazardous Substances & Environmental Engineering. 38: 823-838.

Wang YP, Shi JY, Wang H, Lin Q, Chen XC and Chen YX. (2007). The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter. Ecotoxicology and Environmental Safety. 67: 75-81.

Williams CM, Grimes JL and Mikkelsen RL (1999). The use of poultry litter as co-substrate and source of inorganic nutrients and microorganisms for the ex situ biodegradation of petroleum compounds. Poultry Science. 78: 956-964.