Effect of Composting Process on Some Chemical-Biological Properties and Heavy Metals Behavior of Municipal Sewage Sludge as Affected by Various Bulking Agents

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ABSTRACT: The present study tries to investigate the effect of composting process on some properties and heavy metals status of two municipal sewage sludge (MSS) as affected by three different organic bulking agents (BAs) at three levels (10%, 25%, and 45% V/V). According to the results, the composting process could reduce the fecal coliform to class A, a reduction more obvious in treatments with BAs than produced composts without them. Changes in the chemical properties of the composts vary according to the type of MSS and BAs. Based on the compost quality standard, most of the produced samples are classified in compost-class II. Examination of the total form of three heavy metals (HMs: Zn, Ni, Fe) of both MSS shows that composting process without BAs increases the HMs total concentration, but using of BAs have not been clear trends. In the contrary, BAs application reduced the available form of Fe and Ni, but increased the Zn available form. Examination of chemical forms of each studied HMs also shows that the composting process reduces the Ni and Fe mobility factor, but increases that of Zn. Generally, while, prepared composts can classify into the compost-class II, based on their properties, the high concentration of total Zn above the standard limit makes it extremely restrictive to be used as class II compost. However, according to EPA regulations, the composts with this concentration of Zn can be used as a relatively safe organic material on agricultural land.

Keywords: sewage sludge, stabilization, heavy metals, chemical forms, fecal coliform.

INTRODUCTION

Recent decades have witnessed an increasing production of MSS, which has been hand in hand with the growth of world population. No specific consumption has been defined for them in wastewater treatment plants (Chi et al., 2018). Disposal or safe use of these materials requires a great deal of planning and effort in a way that does not harm public health or cause any environmental problems (Kacprzak et al., 2017). In some parts of Iran, MMS has been used for many years to improve the conditions and fertility of agricultural lands, though its environmental aspects have not been given enough

attention. MSS contains large amounts of organic matter and essential nutrients for plant growth, yet presence of hazardous pathogens, toxic organic compounds and heavy metals in its composition, have associated the application of this organic source in agricultural lands with environmental risks (Udayanga et al., 2018). Therefore, in order to minimize the environmental impact of this source on water, soil, and plants, sewage sludge should be properly stabilized before use (Černe et al., 2019). The processes, undergone for MSS treatment, can modify its properties and make it acceptable for disposal or use in agricultural lands (Černe et al., 2019). Composting is one example of MSS stabilization processes, which converts the substance into stable products (humic substances) through mineralization and humification during biological processes (Chu et al., 2017). It can significantly reduce large numbers of dangerous pathogens of MSS by thermophilic microorganisms (Wang et al., 2018). In sheer contrast to the decrease in the number of pathogens in composting process, numerous researchers have shown that changes in total amounts of HMs in this process does not decrease, but might even increase due to losses of MSS mass and volume (as a result of the dilution effect). However, several studies have shown that the composting process may reduce the bioavailability of heavy metals in MSS (Chiang et al., 2001; Eneji et al., 2003; Zheng et al., 2004). Some studies have suggested adding some materials such as lime, red mud, and coal fly ash so that the availability of HMs in compost could be reduced (Samaras et al., 2008; Liang et al., 2012). Nevertheless, high pH value of such materials has increased the pH of the produced composts, limiting the use of these composts in calcareous soils. Therefore, choosing another additive like organic compounds not only may stabilize the HMs in the compost and via the formation of organic complexes, but can also be used as

bulking agents to improve the mechanical mixing and aerobic conditions (due to high amounts of water and moisture) (Eftoda & McCartney, 2004). The availability of microorganisms to water and oxygen is directly related to total and air-filled porosity. This justifies the use of bulking agents in MSS composting process (Adhikari et al., 2009). Previous studies have shown that the presence of different organic bulking agents, due to increased aeration, reduced NH_3 emissions, and C/N balance, can be effective in the stability of compost organic matter, inactivation of pathogens, and the quality of compost (Iqbal et al., 2010; Ruggieri et al., 2009). Several researches have investigated the effect of different BAs on MSS compost quality (Iqbal et al., 2010; Ruggieri et al., 2009; Banegas et al., 2007). Yet, depending on the characteristics of each MSS in each area as well as composting conditions, more researches on selection of the best BAs and BAs/ MSS ratio are necessary. Organic wastes, including tree leaves, wheat straw, and pistachio hull wastes are heavily produced in Kerman province, Iran. As they have no specific use, utilizing them can both assist the proper management of these wastes, and become a suitable BA in the composting process of MSS. The physical and chemical quality of the bulking agents, used in composting process, can have a significant impact on the quality of produced composts. In addition, it is important to evaluate the properties of produced composts from SS and compare them with compost quality standards, prior to using them in agricultural lands. Having evaluated the quality of compost, produced from SS (from Isfahan wastewater treatment plant, Iran) as affected by different BAs (green plant waste $_{(GW)}$ and dry leaves and pruning waste $_{(PW)}$ with a ratio of 1:1, 1:2, and 1:3 $v_{(SS)}/v_{(BAs)}$), Nezf et al. (2015) showed that the 1:2 and 1:3 mixtures of SS and green/dry plant waste were the most beneficial option for composting, with the final composts attaining high organic matter degradation,

and exhibiting low amounts of heavy metals, a relatively high germination index, and significant reduction of pathogens. Their results showed that addition of BAs had a diluting effect on HMs. The HMs amounts were lower in the 1:3 mixture than the 1:2 one. Malakootian et al. (2014) investigated the possibility of producing compost from sewage sludge (Kerman wastewater treatment plant, Iran) and pistachio hull waste. Their results showed that the properties of the produced compost including temperature, pH, EC, moisture ratio, C/N, P, K, and HMs fell into Category 1 and 2 of the standard compost of Iran.

As for the present study, it aims at investigating the effect of windrow composting process (as a method to recycle MSS and reduce its harmful environmental and health effects) on some chemical and biological properties, as well as the availability of some HMs of two kinds of

MSS, as affected by various baulking agents (tree leaves, wheat straw, and pistachio hull wastes) in different levels.

MATERIALS AND METHODS

In this study, two types of MSS were used for compost production: digested sludge in the digester (D) and dried sludge in lagoons (L). Both had been collected form Kerman wastewater treatment plant (activated sludge with surface aerator), Kerman, Iran. In addition, different kinds of bulking agents, including tree leaves (leaves of broad-leaved tree species and indicated by T), wheat straw (W), and pistachio hull wastes (P) were collected from Kerman province, Iran. Tables 1 and 2 present some chemical properties of MSS and BAs samples, respectively. They were measured via routine methods (Brunner & Wasmer, 1978).

Table 1. Some chemical properties of two studied MSS

Property	Value	Property	Value	Property	Value
D Sample					
pH	6.6	K (%)	1.51	Available-Ni (mg/kg)	1.1
EC (dS/m)	8.13	CEC (cmol(+)/kg)	35.6	Available-Zn (mg/kg)	347.6
OC (%)	19.28	Total-Fe (mg/kg)	14332		
N (%)	2.83	Total-Ni (mg/kg)	18.3		
C/N (%)	6.81	Total-Zn (mg/kg)	1426		
P(%)	1.34	Available-Fe (mg/kg)	336.5		
L Sample					
pH	6.17	K (%)	1.31	Available-Ni (mg/kg)	ND[*]
EC (dS/m)	5.71	CEC (cmol(+)/kg)	52.8	Available-Zn (mg/kg)	421.3
OC (%)	30.94	Total-Fe (mg/kg)	13433		
N (%)	2.51	Total-Ni (mg/kg)	16.8		
C/N (%)	12.3	Total-Zn (mg/kg)	1930		
P(%)	1.72	Available-Fe (mg/kg)	289.3		

ND: Not detected

Table 2. Chemical properties of the bulking agents (BAs), used in the current study

Bulking agents	pH	EC (dS/m)	OC (%)	N (%)	C/N
Tree leaves	7.5	0.6	46.78	0.69	67.80
Wheat straw	6.7	2.6	51.74	0.85	60.87
Pistachio hull waste	7.7	7.7	48.56	2.8	17.34

Table 3. Experimental treatments of produced compost samples and raw MSS (L and D)

Raw L RSS(L)	C of raw L CSS(L)	C of L+10%T T10(L)	C of L+25%T T25(L)	C of L+45%T T45(L)	C of L+10%P P10(L)	C of L+25%P P25(L)	C of L+45%P P45(L)	C of L+10%W W10(L)	C of L+25%W W25(L)	C of L+45%W W45(L)
Raw D RSS(D)	C of raw D CSS(D)	C of D+10%T T10(D)	C of D+25%T T25(D)	C of D+45%T T45(D)	C of D+10%P P10(D)	C of D+25%P P25(D)	C of D+45%P P45(D)	C of D+10%W W10(D)	C of D+25%W W25(D)	C of D+45%W W45(D)

C: Compost; L: Dried sludge in lagoons; D: Digested sludge in digester, T: Tree leaves ; P: Pistachio hull wastes ; W: Wheat straw

A completely randomized experiment was conducted in the form of a factorial design, with three replications, in researching station of Shahid Zendeh-Roh of Kerman (Joopar, Kerman, Iran) in the summer of 2018. To prepare MSS compost samples in each treatment, the bulking agents were added to the MSS (based on the percentage of MSS to bulking agents, equal to 90.10%, 75.25%, and 55.45% V/V) in a layer-by-layer fashion until the stack height became 150 cm approximately. Twenty composts were obtained from mixtures of the BAs and MSS. Table 3 shows the proportion of BAs and MSS (V/V percentages) for each mixture.

Each treatment was 200 cm long, 100 cm wide, and 150 cm high. The humidity (moisture = 70-80%) and temperature was controlled daily during the composting process. Compost aeration was carried out when the temperature reached above 50°C, and continued until the end of the composting process. After 12 weeks, the mature compost samples were collected (3 samples in each treatment) via TMECC method (Test Method for the Examination of Composting and Compost) and transferred to the laboratory. The prepared samples were dried at 60°C (for 72 h) and passed through a 2 mm sieve. Some chemical properties, such as pH, electrical conductivity (EC), total nitrogen (N), organic carbon (OC), and ratio of C/N got measured by conventional methods (Brunner & Wasmer, 1978). In addition, fecal coliforms, as biological properties of the samples, were measured by means of multiple probable number (MPN) 9-tubes method.

In order to evaluate the status of some HMs in the produced compost samples, the total (4M HNO_3-extracted) and available (DTPA-extracted) concentration of Fe, Zn, and Ni were also measured by atomic absorption spectroscopy (AAS: Varian Spectra AA-10).

Based on previous researches' results, evaluation of chemical forms of HMs compared to the available form of each element can have better results than the influence of composting process on status of HMS in MSS. For this purpose, the chemical forms of the mentioned metals were also investigated in some samples (L-samples), using the method of Tessier et al (1979). Table 4 outlines the method. After each successive extraction, the supernatant was collected, filtered, and measured by AAS.

The Duncan's multiple-range test procedure and other statistical analyses were calculated by Microsoft Excel 2010, SAS 9.1.3, and SPSS V19.

Table 4. Summary of the sequential extraction procedure used in this study

g compost : mL solution	Extracting solution	Shaking time (h)	Chemical form of HMs	Symbol
1:8	1 M $MgCl_2$ (pH:7)	1h in 25 °C	Exchangeable	EX
1:8	1 M NaOAc (pH=5 CH_3COOH)	5h in 25 °C	Carbonate- bound	Car
1:20	0.04 M $NH_2OH \cdot HCl$ in 25% CH_3COOH (pH:2 HNO_3)	6h in water bath 95 °C	Bound to Fe/Mn oxyhdroxides	Fe-Mn-OX
1:8	30% H_2O_2+0.02 M HNO_3	2h in water bath 85 °C	Organically- bound	OM
	30% H_2O_2+HNO_3	3h in water bath 85 °C		
	3.2 M CH_3COONH_4 +20% HNO_3	0.5h in 25 °C		
1:8	4 M HNO_3+ 36% HCL	0.5h in water bath 95 °C	Residual	Res

RESULTS AND DISCUSSION

The most important part of each composting process is to reach the optimum temperature (USEPA: at least 55°C for a minimum of 15 days in mechanical turning systems) to disinfect and sterilize dangerous pathogens (Zhu et al., 2004). Figure 1 illustrates the temperature variations of the produced compost samples at different weeks of composting process. It shows that the obtained maximum temperatures from each sample differed based on the types and levels of BAs, as well as the MSS types. In D and L-compost samples, the maximum

temperature variations ranged from 58°C to 63°C (in the 4th to 7th weeks of the composting process) and from 57°C to 64°C (in the 6th to 9th weeks of the composting process), respectively. The maximum temperature in the samples of T(D), P(D), W(D), T(L), P(L), and W(L)-sample belonged to the 7th, 5th, 5th, 8th, 7th, 9th, and 6th-8th weeks of the composting process, respectively. The composting process, itself, displayed four temperature stages, namely mesophilic phase, thermophilic phase, cooling phase, and curing phase (Ishii et al., 200).
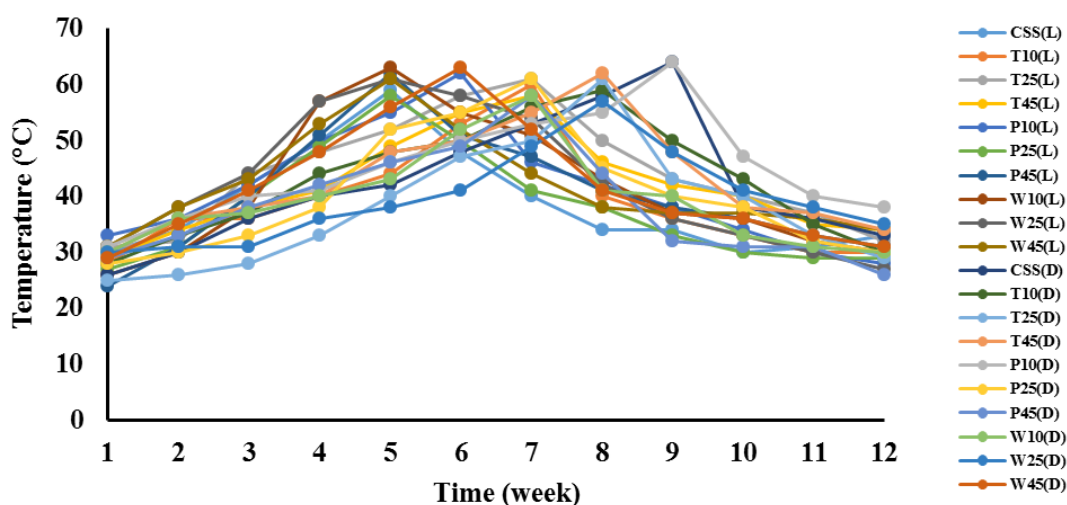

Fig. 1. Variation of temperature in produced composts during composting process (each temperature is a mean of 7 days).

In mesophilic stage, bacteria were active in the temperature ranging between of 20°C and 45°C. Increasing the temperature to 45°C to 75°C would kill the bacteria, replacing them with thermophilic bacteria. Following the thermophilic phase, the compost mass cooled and the mesophilic bacteria became active, and after a short time, the compost reached full maturity, with some actinomycetes, molds, and fungi appearing in the compost (Ishii et al., 2000; Partanen et al., 2010). Based on the results of the present study, in the first three weeks of compost production, the mesophilic temperature (20°C to 45°C) predominated and then the thermophilic phase (45°C to 64°C) appeared. The high summer

temperatures at composting operations location and the measuring of the windrow piles at midday, as well as the heat generated by the organic decomposition microorganisms in the windrow pile, did justify the temperatures of 57°C to 64°C. The presence of such temperatures for 24 hours could eliminate the pathogens. Therefore, with respect to the temperature change, it can be stated that the composting process had been completely done in all samples and based on the type of BAs, changes in the thermophilic process time and the arrival time of the compost in L-samples with BAs were delayed by about 2 weeks, compared to CSS (L)-sample. Yet, in D-samples with and without BAs, the

thermophilic phase remained the same. It was obtained in the 7th and 8th weeks. Vinneras & Jonsson (2002) reported that the use of bulking agents was essential to achieve thermophilic composting conditions. Application of BAs to L-composts (in most cases) increased the thermophilic temperature of the produced composts, though in D-composts, addition of BAs (in most cases) lowered the maximum temperature. Haug (2018) stated that the durability of the thermophilic phase in the composting process depended on both the chemical composition and the adjustment of the parameters, used for the optimal values. Roca-Pérez et al. (2009) in a study of compost production from MSS and BAs of rice straw reported that the thermophilic phase took a long time (for about 15 days) and continued at temperatures above 55°C

for a period of 48 to 144 hours. The study of Tonner-Klank et al. (2007) on fecal compost showed that the use of freshly mown grass in one of the composting containers increased the thermophilic temperature by more than two days in the composting process.

Table 5 shows the values of fecal coliform in some produced composts as affected by different BAs in three time periods. According to the results, the trends of fecal coliform change over time decreased and at the end of composting process period, all samples reached less than 40000 MPN g^{-1} of sludge dry matter. In the samples, produced with different BAs, the amount of fecal coliform was lower than produced composts without BAs (CSSv (L) and CSSv (D)). W10 (L)-sample showed the lowest value of this parameter among the composted samples.

Table 5. Fecal coliform (MPN/g of sludge dry matter) values in produced compost samples during the composting process

	1st day	45th day	90th day
CSS(L)	7.6E+06	6.5E+04	4.0E+04
T10(L)	5.9E+06	4.8E+05	3.3E+03
T45(L)	5.5E+06	4.8E+06	1.9E+03
P10(L)	4.4E+06	6.4E+05	2.3E+03
P45(L)	6.0E+05	2.9E+06	3.2E+03
W10(L)	7.1E+06	5.3E+05	1.7E+03
W45(L)	6.7E+06	3.8E+06	2.9E+03
CSS(D)	8.6E+06	7.2E+06	1.0E+03
T10(D)	7.6E+06	5.3E+05	2.6E+03
T45(D)	5.9E+06	1.3E+06	4.3E+03
P10(D)	7.1E+06	3.9E+05	7.6E+03
P45(D)	5.4E+06	2.9E+06	1.9E+03
W10(D)	6.8E+06	1.4E+06	1.8E+03
W45(D)	1.8E+06	3.2E+05	2.1E+03

Based on the provisions of CFR 40 (Section 503) in EPA standard (1994), MSS is divided into two classes: A and B in terms of fecal coliform values. Class A has a fecal coliform of less than 1000 MPN/g of sludge dry matter, while Class B has a coliform of less than 2 million MPN/g of sludge dry matter. The results of this study showed that at the end of the composting process, the fecal coliform content of the compost sample without BAs ($_{CSS(L)}$) reached less than 40000 MPN g^{-1} of sludge dry matter, which

decreased further in the compost samples, produced with different BAs. Thus, all compost samples would be classified as Class B. However, given that the fecal coliforms of composts produced from BAs ranged below 4000 MPN g^{-1} of sludge dry matter, it would be safe to say that the sludge stabilization process reduced the amount of these pathogens well and could be classified as Class A compost. Moretti et al (2015) in a study of composting sewage sludge with green waste from tree pruning showed that

after 60 days of composting, fecal coliforms declined from 10^7 MPN g^{-1} to 10^4 MPN g^{-1}. Bustamante et al. (2008) reported that high temperatures inside the compost pile could destroy many microbial groups such as total and fecal coliforms, even though the type of waste material used for composting had had a significant effect on the amount of final compost pathogens. Hassen et al. (2001) stated that the thermophilic phase (55-60 °C) changed the bacterial population significantly. They reported the re-growth of escherichia coli of all the windrows mass after the 9th week and the slight secondary growth of streptococci at the end of the composting process.

Evaluation of variance analysis (ANOVA) of the effect of MSS types and different levels of BAs (treatments) on some chemical properties of prepared compost in this study shows statistically significant changes in most properties (Table 6).

Changes in the type of MSS (L or D) on the chemical properties of the produced compost show significant differences in all studied properties (except for N), at 1% level of Duncan test. Also, the effects of different levels of BAs on the chemical properties of produced compost show a significant difference in all chemical properties of the compost (except for pH) at 1% level of Duncan test. The results of the interaction effects of sources of variation on the chemical properties of the compost also show a statistically significant difference in all studied parameters at 1% level of Duncan test.

Figure 2 illustrates the variation of pH values of compost samples in both MSS varieties as affected by BAs.

Table 6. Analysis of variance of some chemical properties of produced composts as affected by types of MSS, BAs treatment, and their interaction.

Source of variation	DF†	Mean square				
		pH	EC	OC	N	C/N
Types of MSS (S)	1	0.0814^{**}	81.3859^{**}	459.1507^{**}	0.0480^{ns}	50.4055^{**}
Treatment of BAs (B)	10	0.0658^{ns}	8.0727^{**}	142.5210^{**}	0.5806^{**}	49.5343^{**}
S×B	10	0.0975^{**}	8.2091^{**}	113.6736^{**}	0.5598^{**}	40.6638^{**}
Error	44	0.0285	0.1970	9.7913	0.1364	6.3487

†Degrees of freedom; *, **Significant at 5% and 1%, respectively; ns: non-significant.
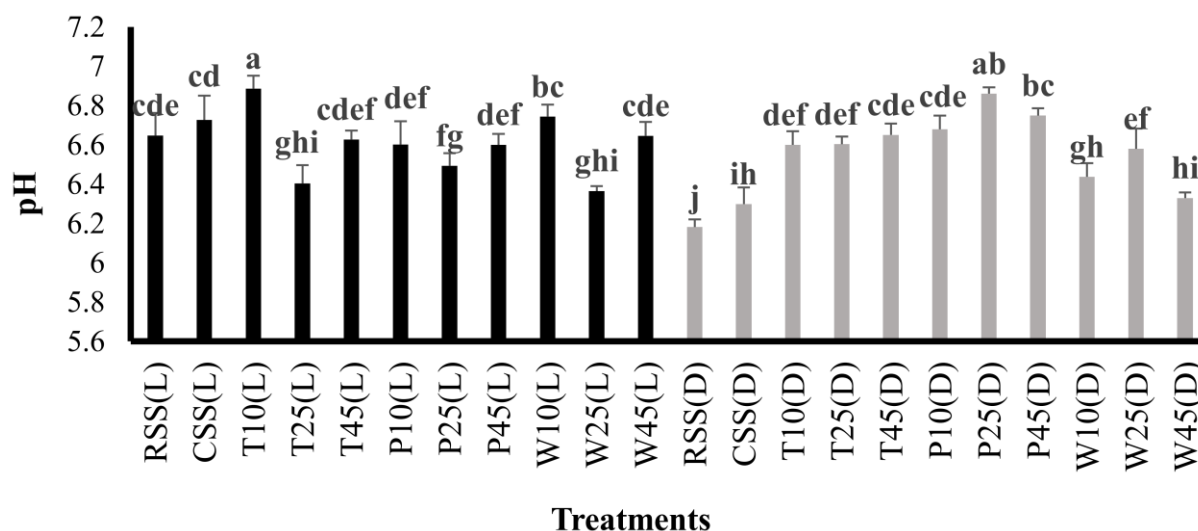

Fig. 2. Variation of pH values in produced MSS composts as affected by various BAs (Differences between the columns with the same alphabet letters are not considerable at 1% level, using Duncan's multiple-range test. Error bars represent standard deviation of three measurements)

According to the results, the effects of the BAs on pH changes varied according to the types of MSS. For example, in some cases of L-samples, the use of BAs reduced the pH, compared to the RSS(L) and CSS (L). On the other hand, application of all BAs at different levels in D-samples greatly increased the pH, compared to RSS(D) and CSS (D). The highest and lowest amounts of pH belonged to T10(L) and W45(D), respectively. The pH values of the produced compost samples ranged between 6.3 and 6.8, complying with the acceptable level of the first class of compost (6 to 8), provided by the Iranian Institute of Standards and Industrial Research (ISIRI, 2011). In the present study, the application of composting process in some L-samples reduced the pH values, which may be due to the production of CO_2 and organic acids by microorganisms (Gunadi et al., 2002). On the other hand, there was an increasing trend in D-samples. Haghighifard Jaafarzadeh et al. (2015) showed that the increase in the pH value of MSS compost was due to ammonia and mineralization of organic nitrogen (as a result of microbial activity), while the decrease in the pH value happened because of nitrification, formation of the ammonium gas, its release to the atmosphere, and eventually release of H ions. (Wong et al., 2001). It seems that the variations in the type of ammonium and nitrate nitrogen in the studied sludge samples have caused the pH changes. As such, it can be said that the presence of more nitrate nitrogen in L-samples decreased the pH, while more organic nitrogen in the D-samples increased it. The difference in pH value in compost samples with different BAs can be due to the varying amounts of lignin and cellulose that are slowly degraded by the bacteria and thus effective in releasing ammonium and nitrate.

According to the results of EC variation (Fig 3), changes in EC values in the production composts did not follow a certain trend.
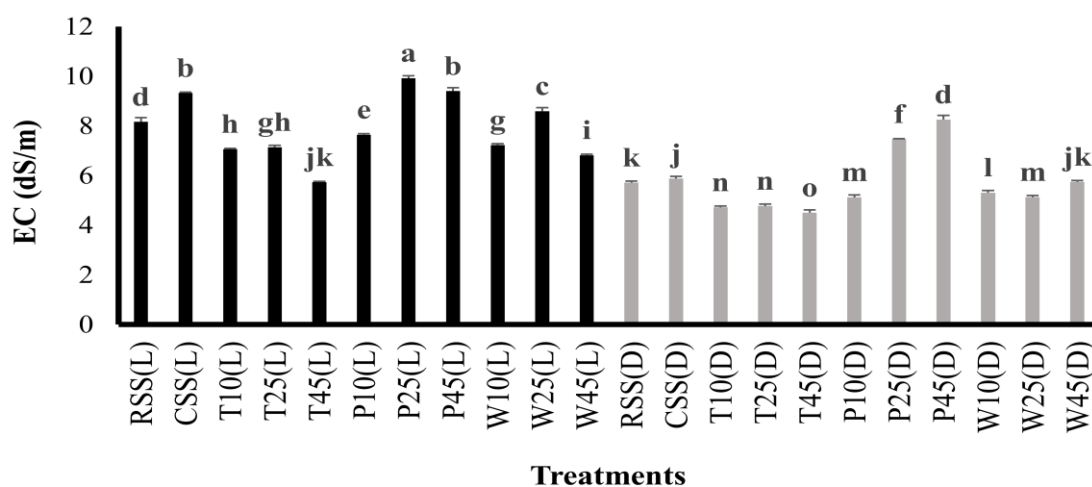

Fig. 3. Variation of EC (dS/m) values in the produced MSS composts as affected by various BAs (differences between the columns with the same alphabet letters are not significant at 1% level, using Duncan's multiple-range test. Error bars represent standard deviation of 3 measurements)

Changes in EC values varied depending on the MSS type, the BAs type, and their levels. High EC values in BAs of P (7.7 dS/m), compared to the other two BAs (2.6 and 0.6 dS/m) is an important reason for increased EC in the produced compost with P. On the other hand, the decrease in EC values in other samples, compared to the un-composted samples, could be attributed to the leaching of soluble salts along with reduced salt content per unit volume of the compost as a result of BAs addition. The highest and lowest values of this parameter belonged to samples P25 (L) and T45 (D), respectively. The decrease in the volume of MSS and the increase in soluble salts per

unit volume of compost were the main reasons for the increased EC values in compost samples without BAs. According to classification of compost quality (ISIRI, 2011), 15 samples of the produced composts were in Class I (below 8 dS/m), with five samples of the produced composts belonging to Class II (below 14 dS/m). Grigatti et al. (2011) showed that the EC of compost samples, produced from MSS, decreased, in comparison to non-composted samples. On the other hand, a research by Tandy et al. (2009) showed that the EC of

sewage sludge increased, compared to non-composted samples as a result of produced mineral compounds in it along with a relative increase in the concentration of ions (decrease in mass of compost due to decomposition of organic matter and also raw materials for composting).

The measured OC content of the produced composts, influenced by the interaction of different levels of BAs and the studied MSS in the composting process (Fig 4), was considerably varied among the treatments.
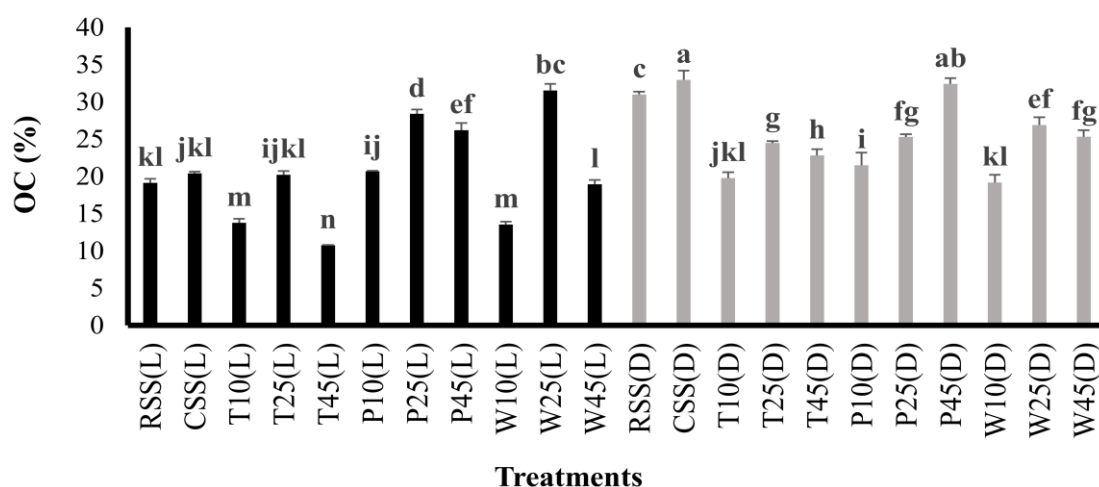

Fig. 4. Variation of OC (%) values in produced MSS composts as affected by various BAs (Differences between columns with the same alphabet letters are not significant at 1% level, using Duncan's multiple-range test. Error bars represent standard deviation of 3 measurements)

In the samples of L, two BAs of P and W increased this parameter, whereas the BAs of T decreased it, compared to RSS(L) and CSS(L). It seems that lower values of the hard fraction of organic matter (lignin and cellulose) of the BAs of T particle caused more decomposition of the compost OC, cutting it in this treatment. On the other hand, in all samples of compost, produced from MSS of D, application of all three BAs at different levels caused this parameter to plummet, compared to RSS(D) and CSS (D). According to the results, the highest and lowest values of this parameter between produced composts were observed in P45(D) and T45(L) samples, respectively. Furthermore, the OC values in the produced compost samples ranged from 10.73% to

33%. Given the acceptable level of OC (i.e., Class I: above 25% and Class II: above 15%), provided by ISIRI (2011), the values obtained for the majority of samples were classified in Class II of compost. Hernández et al. (2006) in their study on the production of sewage sludge compost with different BAs showed that the large amount of organic matter was reduced via mineralization of many labile components by microorganisms. Khalil et al. (2011) analyzed OC depletion within the composting process and expressed its conversion to carbon dioxide. Diaz et al. (2007) showed that about 30 to 60% of the organic matter got decomposed by the composting process. A study by Gao et al. (2015) illustrated that in the composting process, the amount of organic matter

declined due to mineralization and production of humic substances.

According to the results of total N in prepared samples, the composting process in most cases reduced this parameter, compared to the non-composted samples. This was justified by the process of nitrate denitrification and sublimation of ammonium to ammonia at a high temperature during the composting process; however, the trend of N changes in the treatments did not follow a specific order based on the kinds and levels of BAs (Figure 5). According to the results, the highest amount of this property was in the presence of P25(L). Also, the N content in the produced compost samples ranged between 1.21% and 3%. Thus, given the acceptable level of N content (i.e., Class I: 1.25% to 1.66% and Class II: 1% to 1.5%) of compost, provided by ISIRI (2011), these values stood above the minimum value of 1.25 %, qualifying the compost as Class I.

Results from the effects of different BAs and MSS levels on C/N ratio of the produced compost (Fig 6) show that without BAs (CSS(L) and CSS(D)) composting process raised this ratio, in comparison to the un-composted samples (RSS(L) and RSS(D)). The trend of changes in this ratio was not marked by the use of BAs, yet only with increasing levels of P application, was a considerable increase observed in the ratio of C/N in the compost, produced in both MSS.
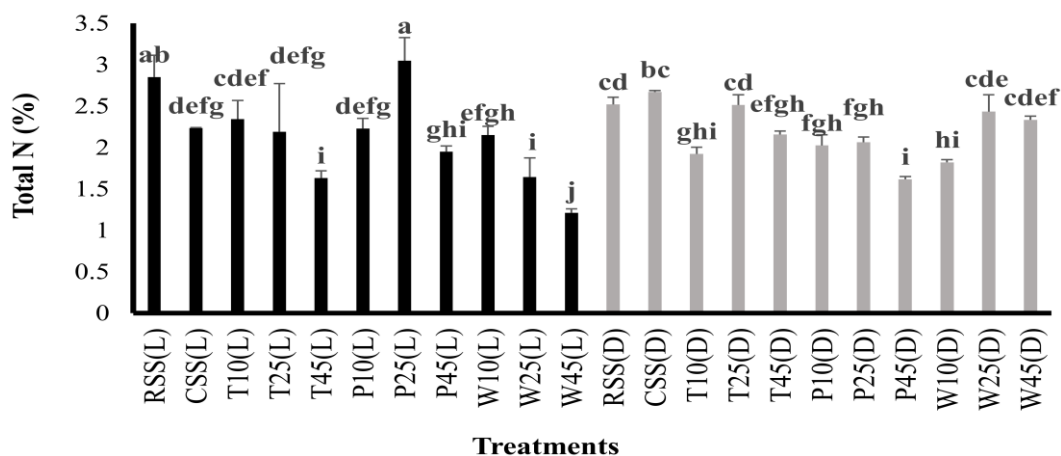

Fig. 5. Variation of total N (%) values in produced MSS composts as affected by various BAs (Differences between columns with the same alphabet letters are not significant at 1% level, using Duncan's multiple-range test. Error bars represent standard deviation of 3 measurements)
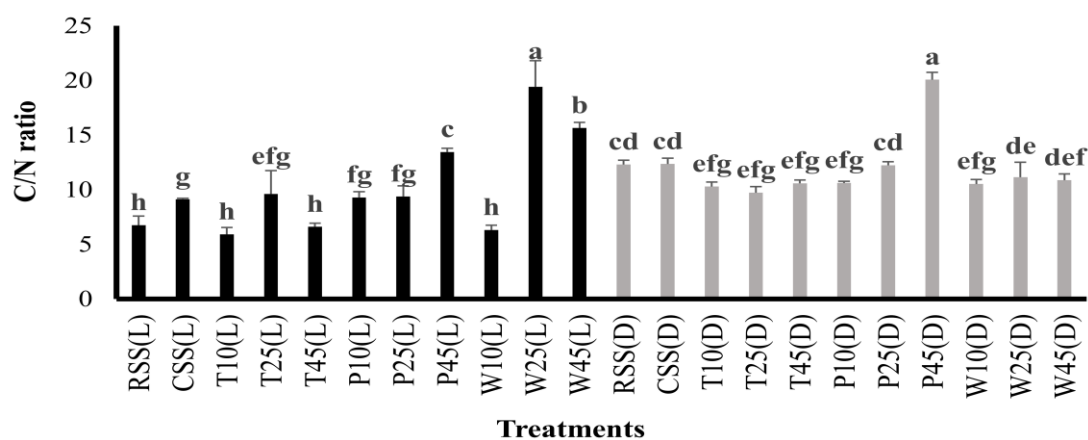

Fig. 6. Variation of C/N ratio in produced MSS composts as affected by various BAs (Differences between columns with the same alphabet letters are not significant at 1% level, using Duncan's multiple-range test. Error bars represent standard deviation of 3 measurements)

Depending on the MSS type, the composting process would both increase and decrease the C/N ratio in the L and D-samples, respectively. The highest and lowest levels of this parameter were observed in samples P45 (D) and T10 (L), respectively. Based on the results, the C/N ratios in the produced compost samples ranged from 5.9 to 20. Given the acceptable range of C/N ratio (i.e., Class I: 15-20 and Class II: 10-20) in composts, the majority of compost samples fell under Class II. Gao et al. (2015) found out that during the composting process, carbon would be converted to carbon dioxide and humus, while the nitrogen source would be partially released as gas or would be converted to nitrite and nitrate through nitrification, thence to be absorbed by living organisms. Ultimately the C/N ratio would decrease. In the present study, in some compost samples,

this ratio showed an increasing trend, likely due to the presence of different carbon sources of the BAs.

Results from ANOVA showed that both total and available concertation of Fe, Ni, and Zn were greatly influenced by the MSS types, various BAs kinds of, and their interaction (Table 7).

Tables 8 and 9 show the total and available concentration of Fe, Ni, and Zn in MSS samples as well as the produced compost with/without BAs application, after 12 weeks. A comparison of total concertation in RSS (L and D) with CSS (without BAs) samples shows that the composting process, due to losses of SS mass (biodegradation of organic matter), increased the total concentration of the mentioned HMs in CSS. These findings agreed with previous studies (Cai et al., 2007; Zorpas et al., 2008).

Table 7. Analysis of variance of total and available form of Zn, Ni, and Fe of the produced composts as affected by MSS types, BAs treatment, and their interaction

Source of variation	DF†	Mean square					
		Total form			Available form		
		Zn	Ni	Fe	Zn	Ni	Fe
Types of MSS (S)	1	2902386.40**	276.70**	36753633.4**	39298.4449**	0.2305**	1379.683**
Treatment of BAs (B)	10	116661.11**	36.15**	24985948.0**	2276.4773**	0.1645**	7548.024**
S×B	10	216390.42**	40.92**	20299860.3**	3909.6477**	0.1676**	4304.79888**
Error	44	16291.4510	5.8956	2599633.4	426.2905	0.0306	171716.3100

†Degrees of freedom; *, **Significant at 5% and 1%, respectively; ns: non-significant.

Table 8. Total concentration of Fe, Ni, and Zn (mg/kg) in raw MSS$_s$ and their produced compost samples

Samples	Fe	Ni	Zn
RSS(L)	14318.67±39.8	17.17±1.3	1430.67±15
CSS(L)	18032.67±4.7	20.19±1.8	1660.95±69.8
T10(L)	21231.67±14.5	18.71±2.2	1834.33±12.5
T25(L)	19851.33±53.3	16.84±1.9	1631.67±145.1
T45(L)	19220.67±27.8	16.87±1.2	1588±83.5
P10(L)	18329.67±52.4	19.24±0.5	1984.33±10.7
P25(L)	17980.67±123.1	13.88±0.4	1633.9±21.1
P45(L)	16579.67±203.6	14.12±0.5	1532.33±8.1
W10(L)	22715.33±214.5	13.85±0.2	1980.33±19.4
W25(L)	21348±193.6	14.43±1.9	1843±29.6
W45(L)	20359.67±428.9	12.16±1.6	1787.67±38.2
RSS(D)	13431.73±40.7	16.88±2.2	1929.67±27.5
CSS(D)	16351.33±30.7	18.28±0.4	2301±45.7
T10(D)	17851.67±65.5	27.4±0.5	2376±288
T25(D)	16350.33±48.9	24.68±0.3	2276.33±212.8
T45(D)	14277±28.9	21.25±1.4	2118±18
P10(D)	22609.33±15.5	18.77±0.6	2036.33±43.1
P25(D)	20378.33±17.8	16.96±0.6	1987±11.1
P45(D)	18359.33±52.5	17.41±1.5	1968.33±39.7
W10(D)	19376.33±22.7	23.52±2.8	2231.33±156.7
W25(D)	18777.67±43.2	19.82±2.1	2197.33±65.7
W45(D)	15787.67±9.6	17.54±2.2	2099.33±42.5

According to Table 8, application of each BAs in different volumes had dissimilar impacts on HMs concentration in the composted samples. For instance, application of 10% of each BAs increased the total Zn concentration, compared to CSS, whereas application of 45% BAs (with the exception of W45) reduced the total amount of Zn. Seemingly, in lower levels of BAs, preparation of suitable aeration conditions may improve the composting process, decreasing the MSS mass and consequently increasing the HMs concentrations, unlike the CSS. On the other hand, higher levels of BAs would decline the HMs concentration in composted MSS,

which is due to the dilution effect. The changes of total concentration of Fe and Ni, as affected by BAs, had an unclear trend; however, in general the total concentration of Fe increased. The total Ni content in the produced compost samples ranged from 12 to 27.5 mg/kg. This was in accordance with the acceptable level of Ni in Class I compost (maximum 120 mg/kg), provided by ISIRI (2011) and the permissible limit for application of sewage sludge to agricultural land (420 mg/kg), based on the EPA standard (1994). The obtained values fell well below the standard limit for both suitable compost and direct use of MSS for agricultural land.

Table 9. Available form of Fe, Ni, and Zn (mg/kg) in raw MSS$_s$ and their produced composts samples

Samples	Fe	Ni	Zn
RSS(L)	336.5±6.89	1.1±0.05	347.6±9.37
CSS(L)	323.6±4	0.2±0.01	319.74±9.99
T10(L)	287.3±1.29	ND	380.94±2.04
T25(L)	243.6±1.54	ND	346.6±6.41
T45(L)	217.63±0.84	ND	331.54±3.3
P10(L)	234.6±1.46	ND	332.37±12.51
P25(L)	218.5±3.35	ND	321.6±11.85
P45(L)	198.6±5.86	ND	319.6±6.95
W10(L)	307.6±2.55	ND	336.7±11.57
W25(L)	291.6±3.84	ND	314.58±8.8
W45(L)	272.6±8.65	ND	308±9.32
RSS(D)	289.3±7.42	ND	421.3±14.39
CSS(D)	301.3±6.1	ND	403.1±3.69
T10(D)	273.3±5.2	ND	376.5±3.54
T25(D)	243.6±1.35	ND	353.1±10.09
T45(D)	237.8±6.99	ND	345.2±5.89
P10(D)	283.6±13.59	ND	386.5±5.18
P25(D)	268.8±7.04	ND	377±5.56
P45(D)	233.3±4.58	ND	325.2±8.6
W10(D)	323.65±6.67	ND	431±8.94
W25(D)	2906±67.04	ND	397±0.97
W45(D)	287.6±19.67	ND	380.2±4.53

ND: Not detected

On the other hand, in contrast to Ni amounts, total Zn values in the produced compost samples ranged from 1530 to 2376 mg/kg. Based on the acceptable level of Class I compost (maximum 1300 mg/kg), provided by ISIRI (2011), the values stood above the standard limit as a suitable compost. However, due to lower values than the permissible limit for direct application of sewage sludge to agricultural land (2800 mg/kg), presented by EPA standards (1994),

they were suitable for direct use on agricultural land. Interaction effects of different BAs and MSS levels on the available form of Fe and Zn (Table 9) showed that composting process decreased the amount of available form of these elements in most treatments. As the BAs levels rose, the available amounts of these elements dropped further. This could be attributed to the formation of stable forms of these elements with different BAs portions

(form of organic complex or bound to iron oxides). On the other hand, the available Ni values showed the produced composts (except for the compost, produced without BAs). The available amounts of this element was negligible and indistinguishable by the AAS. The available Ni content of compost, produced without BAs, also showed a

significant decrease in this form of Ni, compared to the MSS of L.

According to the results of ANOVA, the composting process with different BAs had a strong effect on all chemical forms of Ni, Fe, and Zn at 1% level of Duncan test (Table 10).

Table 10. Analysis of variance of chemical forms of Zn, Ni, and Fe of produced composts as affected by composting process

Source of variation	DF†	Mean square				
		Ni				
		EX	Car	Fe-Mn-OX	OM	Res
Treatment	10	0.4132[**]	3.1199[**]	1.3223[**]	0.2104[**]	10.1782[**]
Error	22	0.0033	0.0054	0.0129	0.0244	0.0477
		Fe				
Treatment	10	14185.7076[**]	8983.5137[**]	1080041.96[**]	2026388.45[**]	7086819.56[**]
Error	22	72.4794	45.0871	9857.88	447.83	14532.88
		Zn				
Treatment	10	1673.4049[**]	3275.0795[**]	10481.1045[**]	7154.2862[**]	9804.9192[**]
Error	22	24.5227	68.2055	93.2052	152.1678	290.0233

†Degrees of freedom; *, **Significant at 5% and 1%, respectively; [ns]: non-significant.

Figure 7 shows the chemical fractions of Ni in composted samples and RSS(L). According to the results, the forms associated with Fe-Mn-OX (29%) and EX (10.5%) had the highest and lowest amount of Ni chemical forms, respectively. The compost, produced from RSS(L) resulted in an enormous increase in the Res (48.6%) form and a decrease in EX (4.1%), Car (5.5%), and Fe-Mn-OX (20.7%). Hanc et al. (2012) showed that composting process on kitchen and garden wastes reduced the EX and oxidizable fractions of Ni, yet increased the Res form. Zheng et al. (2007) reported that the highest concentration of Ni in MSS existed in OM form, followed by Car, and Fe-Mn-OX. They showed that with composting process the EX, Car, Fe-Mn-OX, and OM fractions of Ni were transformed to Res fraction. The trend of variations in chemical forms of Ni in composted samples via BAs were similar to CSS(L), compared with RSS(L), even though the results indicated a decrease in Car (1-4.7%) and Res (33.6-44.6%) contents and an increase in other forms, compared to the CSS(L).

Based on the results obtained from the Ni mobility factor (Figure 8), composting of RSS(L) (without BAs) reduced the mobility of Ni from 31.3% to 9.6%. Also, the use of different BAs in composting process showed a considerable decrease in mobility factor of some samples, compared with CSS(L). Among the samples, the W45(L) displayed the lowest mobility factor (5.25%). Generally, BAs application inhibited transformation of predominant Fe-Mn-OX form into mobile fractions during the composting process. Investigating the chemical forms of Fe (Figure 9) in RSS(L) showed that most of this element was in two forms: Res (59.2%) and Fe-Mn-OX (32.9%). On the contrary, other forms of Fe in RSS involved a small part of this element (>8%). The process of composting (CSS) caused a slight change in chemical forms of Fe, which was accompanied by an increase in OM (9.8%) form and a decrease in three forms of Fe-Mn-OX (27.4%), Car (1.71%), and EX (1.6%). The use of BAs, like CSS(L) results, was accompanied by an increase in OM form, while reducing the other forms. However, both the reduction in

the form of EX and the rise in OM (12.3-20.5%) form in these samples were remarkable. The process of composting decreased the Fe mobility (Figure 8) in all produced compost samples, in comparison with RSS (4.4%), the highest decrease of which was observed in P45 (2.27%).
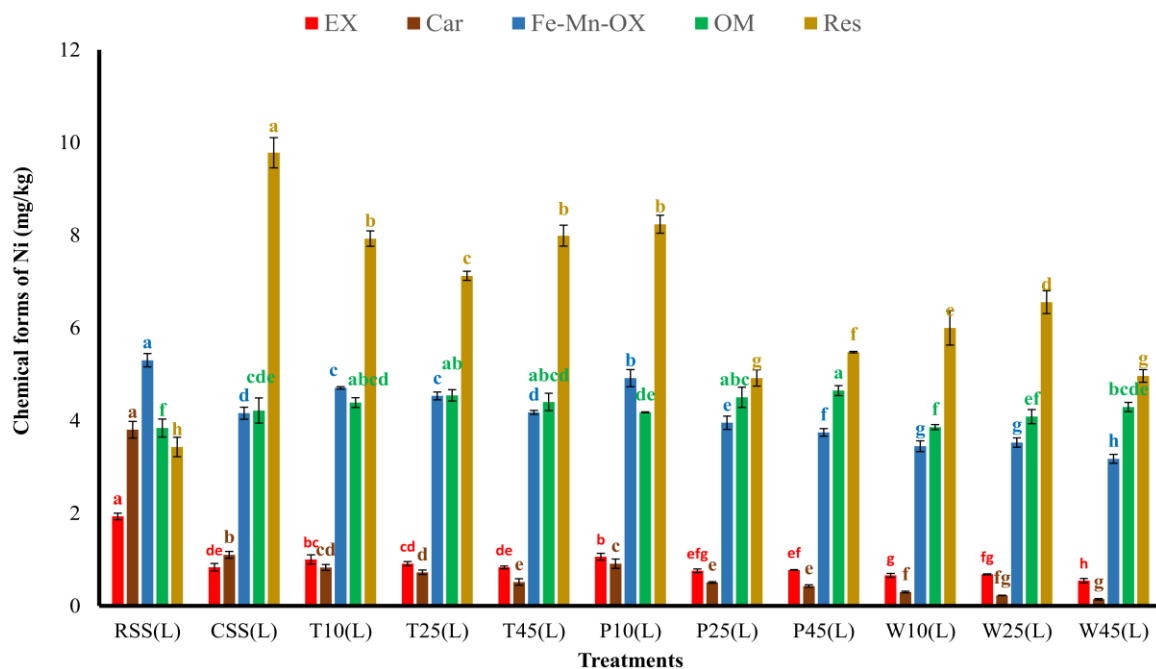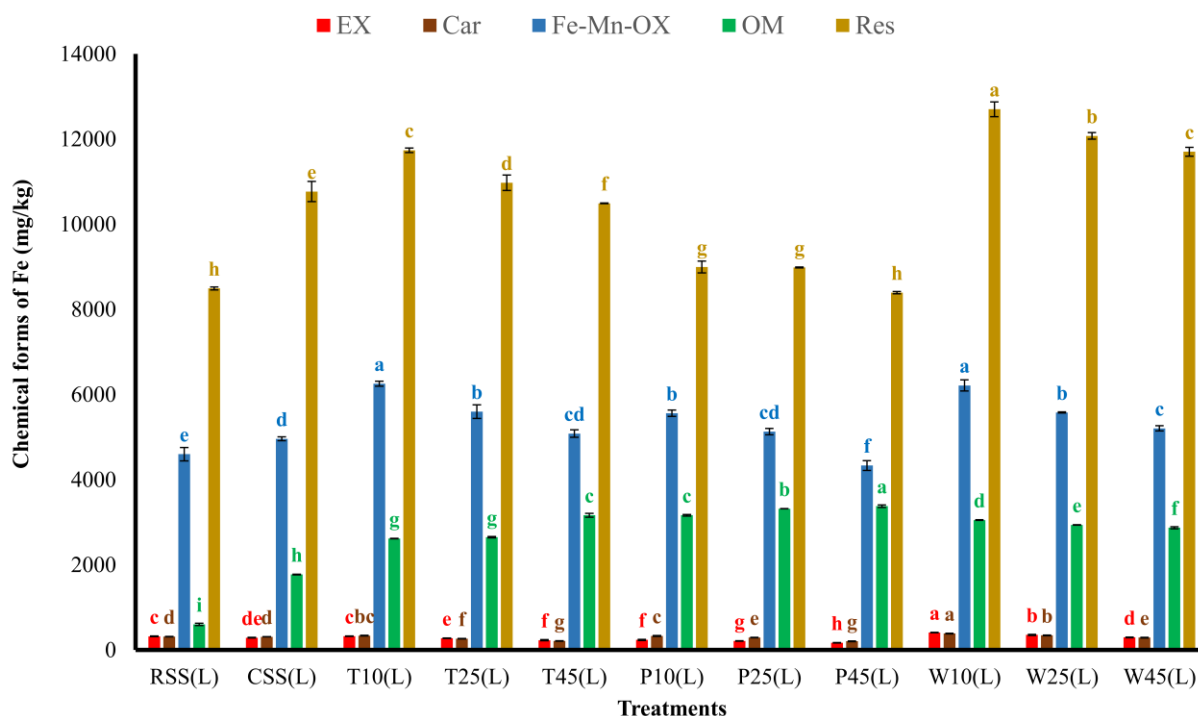

Fig. 7. Chemical forms of Ni (mg/kg) in RSS (L) and its composted samples (Differences between columns in each chemical form with the same alphabet letters are not significant at 1% level, using Duncan's multiple-range test. Error bars represent standard deviation of 3 measurements)

Fig. 8. Mobility factor of Ni, Fe, and Zn in RSS (L) and its composted samples

Fig. 9. Chemical forms of Fe (mg/kg) in RSS (L) and its composted samples (Differences between columns in each chemical form with the same alphabet letters are not significant at 1% level, using Duncan's multiple-range test. Error bars represent standard deviation of 3 measurements)

The Zn chemical forms (Figure 10) in RSS(L) showed equal and high concentration of Res (27.7%), Fe-Mn-OX (26.1%), and OM (26%) forms, in comparison to other forms of EX (3.2) and Car (16.8%). In a study on chemical forms of HMs in MSS, Weng et al. (2014) showed that the acid-soluble/exchangeable and reducible fractions had the highest amount of Zn in the MSS, increasing the mobility factor of this element. Fuentes et al. (2006) examined the chemical forms of Zn in an anaerobic stabilized sludge, showing that the Ex (28%) and reducible fractions (20.5%) had the most forms of Zn. The process of composting in CSS(L) reduced the Res (17.5%) form, increasing other forms, especially EX (6.2%), compared to RSS. Chiang et al. (2007) and Nomeda et al. (2008) reported that due to

oxidation of organic matter during the composting process of Zn as well as its high potentiality to reduce oxidation, the mobile fractions of this metal rose (the OM and Res forms could be transformed into EX form). Cai et al. (2007) in their study on composting effect of SS on Zn chemical forms showed that the Res forms were converted to Fe-Mn-OX and OM forms. Application of different BAs in composting process, increased all chemical forms of Zn in MSS, except for Res, which was decreased (16.1-21.5%). Contrary to other studied elements, the process of composting with and without various BAs increased the mobility factor (Figure 8) of the Zn, compared to the RSS(L) (20.1%). Maximum and minimum mobility factors belonged to T25(L) (24.85%) and CSS(L) (24%), respectively.
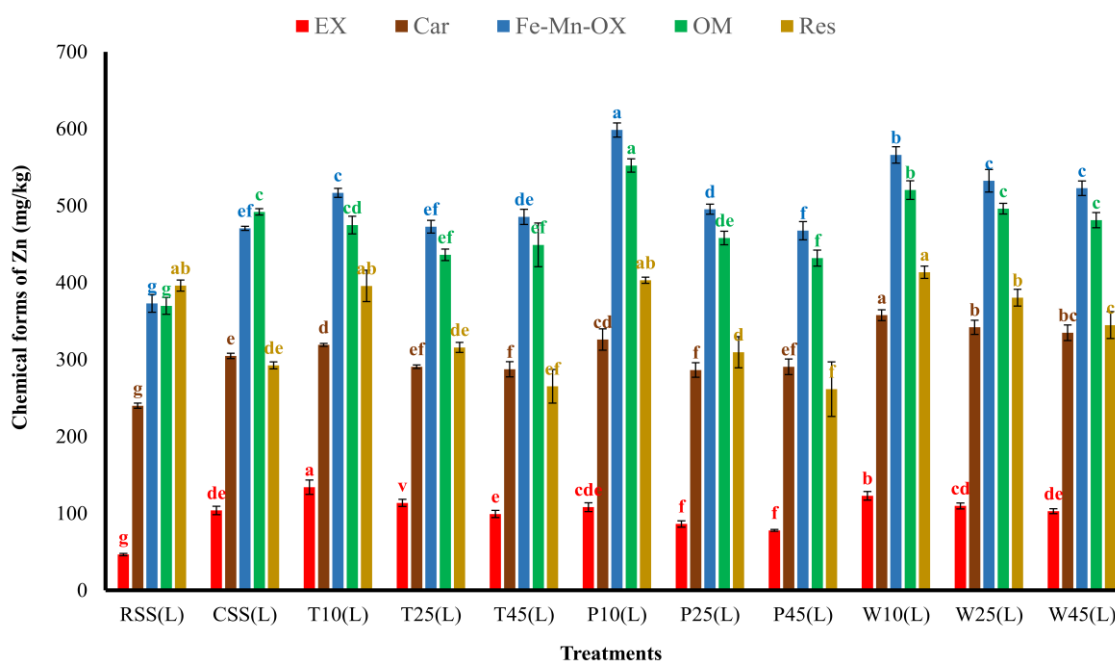
Fig. 10. Chemical forms of Zn (mg/kg) in RSS (L) and its composted samples (Differences between columns in each chemical form with the same alphabet letters are not significant at 1% level, using Duncan's multiple-range test. Error bars represent standard deviation of 3 measurements)

CONCLUSION

According to the results from this study, composting process could reduce the fecal coliform pathogen through MSS stabilization. Here, application of BAs (especially W) decreased more pathogens than composted samples without BAs. The composting process increased the pH values of MSS samples. This trend was obvious in samples with BAs. The changes in the EC of the MSS were strongly influenced by the volume of BAs, qualifying the compost to belong to Class II composts. Organic carbon dropped in most treatments during the composting process, but did not cause the produced compost to be disqualified from Class II compost. In addition, even though the composting process reduced the N content of MSS, the final amounts of N in the produced composted got classified in Class II compost. Changes in the total amounts of the three heavy elements of Fe, Zn, and Ni rose. This was due to the composting process, itself, though the elements' available forms (with the exception of some Zn treatments) decreased.

Investigation of the chemical forms of these three elements showed a decrease in the mobility factor of Ni and Fe along with an increase of this factor in Zn as a result of the composting process. Overall, the composting process in both MSS reduced both the pathogens and HMs availability well. Therefore, the composting process could be improved by applying all BAs, studied in the present research, especially the ones at 45% levels.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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