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The objective of the *Journal of Residuals Science & Technology* 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 and are also welcome.

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TECHNICAL NOTE

Prediction of Microbial Inactivation in Biosolids as a Function of Moisture Content and Temperature using Artificial Neural Networks

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ABSTRACT: Agricultural land application of biosolids is an environmentally sound method for recycling stabilized sludge generated during wastewater treatment processes. Meeting regulatory microbial criteria remains an expensive and time consuming process, especially when testing is performed prematurely and requires re-testing. The objective of this study was to determine how well neural network monitoring predicted microbial survival in response to changes in temperature and moisture content of biosolids under laboratory conditions. The backpropagation feed-forward neural network method showed superior performance in predicting the microbial inactivation of fecal coliforms ($R^2 = 0.95$) as a function of temperature and moisture relative to linear-regression models. Although mathematical modeling may not be a sufficient substitute for microbial testing, it may be a useful support tool to optimize the timing of testing for microbial quality designation of biosolids. Further studies involving prediction of specific pathogen levels (*Salmonella sp.*, enteric virus, and viable helminth ova), while incorporating a variety of sludge matrices and environmental parameters, are needed to help determine the full advantages of ANN modeling.

INTRODUCTION

LAND application of biosolids has been widely practiced in North America for many decades. In the USA, over 50% of the 6 million metric tons (dry) of the biosolids produced are annually applied to the land as class B biosolids, which have been treated to significantly reduce pathogens to levels accepted for land application [1]. Land application is attractive due to its nitrogen, phosphorous, and organic matter content which benefits plants and soil. Heavy metals and pathogens that may exist in biosolids have led to restrictions and public apprehension for land application. Testing must be performed in order to determine the classification type of the biosolids and the subsequent disposal restrictions [1]. Tests can be expensive and must be repeated if the established chemical and microbial limits are exceeded, or if distribution does not take place within a suitable timeframe [2].

Many studies have focused on the accurate prediction of microbial inactivation [3]. Accurately predicting microbial levels in biosolids can help minimize repeated microbial analysis due to testing too early in the process or too early after a rainfall event, resulting in time and monetary savings. Inactivation studies quantify the effects of interactions between two or more factors and allows interpolation of combinations that have not been tested explicitly [4]. Most studies were dependent upon statistical tools that simplify interactions into uncomplicated equations such as inactivation rates as a function of a single variable, thereby limiting their accuracy [5, 6].

Statistical models expressed in the Artificial Neural Network (ANN) format include a number of regression, discriminant, density estimation and graphical interaction models [7]. Although some ANN models are not significantly different from a number of standard statistical models, they are extremely valuable as they provide a flexible means for implementation. In addition, unlike some statistical models, ANN models can be extended easily from univariate to multivariate cases and non-linearities can be incorporated effortlessly. Recent studies have adopted ANN models to

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solve multivariate and complex problems in microbiology, biochemistry, biology, molecular biology and genetics [8, 9].

Neural networks are trained using a learning rule by presenting the network with training data. One of the most widely used learning processes is a back-propagation training algorithm (Figure 1), which propagates the information backwards and forwards several times through the neural network in order to update the weights of the links connecting the neurons and minimize the error between the observed and computed outputs [3].

Errors in the output determine measures of hidden layer output errors, which are used as a basis for adjustment of connection weights between the pairs of layers and recalculating the outputs. This iterative process is carried out until errors fall below a tolerance level. Learning rate parameters scale the adjustments to the weights. A momentum parameter can also be used to scale the adjustments from a previous iteration and adding to the adjustments in the current iteration [10]. ANN models were applied in this study to investigate whether they could accurately predict microbial inactivation mechanisms in biosolids under varying levels of moisture and temperature combinations. Results from the ANN models were also compared to simple linear regression models.

MATERIALS AND METHODS

Description of Experimental Setup

Solar dried, aerobically digested and belt-pressed biosolids were collected from the Green Valley Wastewater Treatment Plant (Pima County, Arizona). Samples containing 10g of solar dried biosolids were placed into sterilized Nalgene jars and sterile distilled water was added to adjust the moisture content to 10%, 35%, 65%, and 80% (g water/g wet sample). Twenty-four samples were prepared for each moisture content level to provide for three replications of sampling on 9 distinct sampling dates taken over time. Each jar was then inoculated with 1.0 mL of *Escherichia coli* ATCC 15592 (*E. coli* 15592) obtained from the Department of Soil, Water and Environmental Science, University of Arizona. All jars were then placed in a three-shelf convection oven (Isotemp vacuum oven 282A, Fischer Scientific International, Inc., Hampton, N.H.) at set point temperatures of 25, 32 and 38°C. Thermocouples were placed at the front and back of each shelf to continuously monitor temperature gradients throughout the experiment. The selected set point temperatures were based on a range of average heat units measured during the warmer field drying experiments conducted at the Green Valley Waste Water Treatment Plant (GVW-

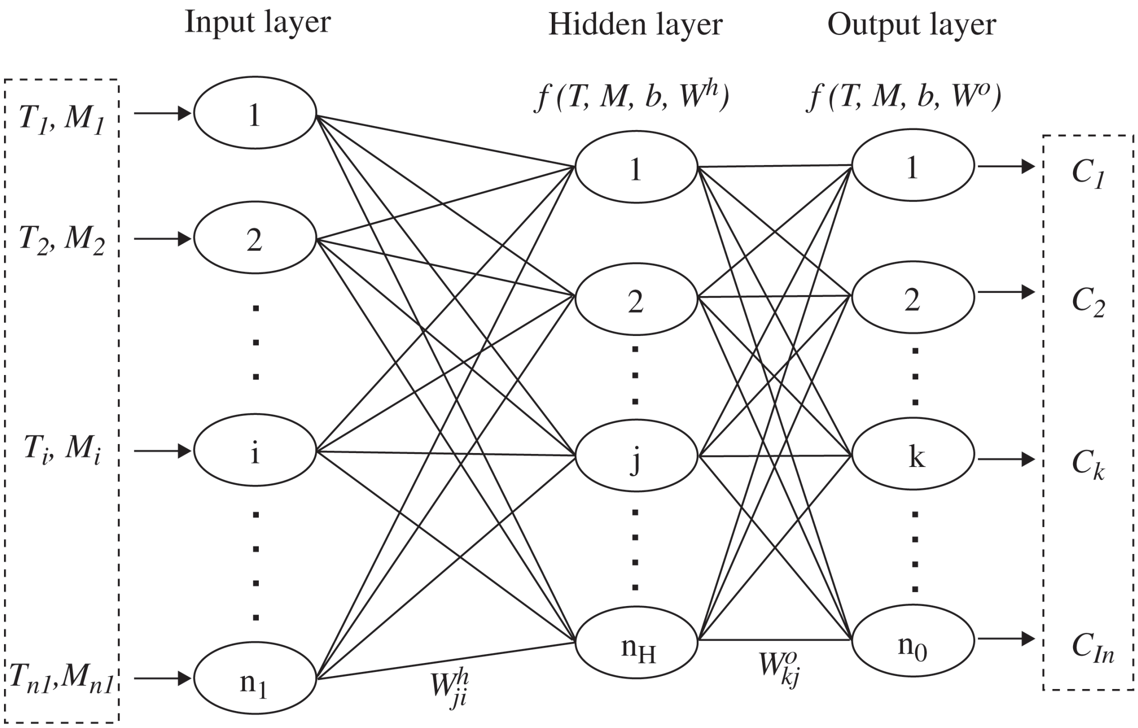


Figure 1. *Schematic structure of how a neural network works. $T_1 - T_{n1}$: input temperature values provided through an input layer; $M_1 - M_{n1}$: input moisture level values provided through an input layer; W^h_{ji} and W^0_{kj}: weights involved in computation in hidden and output layer; b: bias; and f: non-linear activation or transfer functions.*

WTP), Green Valley, Arizona (unpublished data, Choi and O'Shaughnessy, 2004-2005). Two thermocouples, relative humidity and temperature sensors (CS500 RH, Campbell Scientific, Logan, Utah, USA) were placed on the top, bottom, and middle racks of the convection oven.

Microbial Sampling and Assay

The inoculum was prepared by isolating a single colony of *E. coli* 15592 from a TSA (Tryptose Soy Agar) plate and inoculating a 100 mL flask of Tryptose Soy Broth (TSB) for approximately 18 hours in an incubator at 38°C. The initial titer levels were enumerated by plating and dilution on mEndo Agar; the approximate titer levels at the time of inoculation were $\log_{10}$ 8 CFU/mL. The Nalgene jars were weighed prior to packing the biosolids and again prior to sacrificing the jar when assaying the samples to determine if any significant amount of moisture was lost. A total of seven sampling days were scheduled for each temperature run and three replicates of each moisture level were prepared.

The dilution and plating method was used to enumerate fecal coliforms for the moisture levels samples prepared at the 10%, 35%, 65% and 80% levels. The dilution solution was comprised of sterile K_2PHO_4 and agar utilized for plating was mFC agar (Acumedia, Baltimore, MD, USA). At the 10% moisture level, the three-tube Most Probable Number (MPN) method [11] was used in addition to the plating technique to obtain fecal coliform readings below $\log_{10}$ 2. The MPN method is particularly useful for low concentrations of organisms (<100/g) especially for those samples whose particulate matter may interfere with accurate colony counts [12, 13]. Titer levels on Day 0 of the experiments were determined by plating dilutions of the inoculum on m-Endo Agar (Difco Co., Detroit, MI, USA) and were determined to be in the range of $\log_{10}$ 8 CFU/ml and $\log_{10}$ 9 CFU/ml.

Model Development: Backpropagation

In any model development process, familiarity with the available data is of the utmost importance. ANN models are no exception, and data pre-processing can have a significant effect on model performance [14]. To describe the microbial responses to environmental stresses over time, the microbial inactivation rate (k_d) was calculated by the first-order kinetic equation, $k_d = -\log(N_0/N)/t$, where N_0: initial number of *E. coli*, N: final number of *E. coli*, and t: time [15]. A total sample size of 144 was used for each temperature level. A total of 36 microbial inactivation rates calculated by the first order kinetic model (called as k_{d_Exp}) were used for ANN computation. Experimentally calculated inactivation rates (k_{d_Exp}) were then compared to ones predicted by ANN (called as k_{d_ANN}).

Regarding data pre-processing, all data used in the calculations were normalized within a uniform range (0–1) to prevent larger numbers from overriding smaller ones, and to prevent premature saturation of hidden nodes which impede the learning process. Networks trained with experimental data that adequately represent the overall characteristics of the critical physical processes will achieve higher generalization ability. A total of 36 data points used in this study were divided into three subsets: a training set (72% of the total), a validation set (8% of the total), and a test set (20% of the total). In this study, the k-fold cross validation method was used to improve the generalization of the network and prevent over-fitting [16]. All data were randomly divided into $k = 9$ subsets of equal size and different network architecture and selected parameters were identified and trained. Each time one of the subsets was left out during the training the omitted subset was used for validation. This procedure was repeated until no further decrease in error occurred among 9 subsets. After training and validation, the network architecture having the smallest error over 9 subsets was selected and then evaluated using the test set.

In most function approximation problems, a neural network with one hidden layer can approximate functional continuous mapping provided the number of hidden neurons is sufficiently large [16]. Therefore, the number of hidden layer was fixed to one in this study. Learning coefficient ratio and momentum terms were commonly used in weight updating to help the search escape local minima and to reduce the likelihood of search instability. This accelerated the weight updates when there was a need to reduce a backpropagation learning rate to avoid oscillation. The transfer function was necessary to transform the weighted sum of all signals impinging onto a neuron so as to determine its firing intensity [17]. Responses of the model to a range of learning coefficient ratios, momentum and transfer functions were assessed.

Governing factors on ANN computation were evaluated to achieve the best architectural performance for the neural network. These factors included the effects from the number of iterations, momentum, learning coefficient ratio, transfer function (linear, TanH, sigmoid, DNNA [Digital Neural Network Architecture] and sine) and the learning rate rule (Delta-rule, Normalized cumulative delta, Extended Delta-Bar-Delta, Quickpro, Maxpro and Delta-bar-delta). NeuralWorks Professional II/Plus (NeuralWare, Inc., Pittsburgh, PA) running in a Windows environment was used for computation in this study.

RESULTS AND DISCUSSION

Average measured temperature for each shelf was 23.3, 25.0 and 26.7°C at the 25°C set point; 29.6, 32.0 and 34.4°C at the 32°C set point; and 35.9, 38.0 and 40.1°C at the 38°C set point. Data averaged from the thermocouples placed at each shelf level indicated an average temperature gradient of ± 2.1°C from the middle to the top and bottom shelves, respectively.

Complex Dynamics of Fecal Coliforms in Biosolids

Effects from temperature and moisture level on *E. coli* survival in biosolids were examined using analysis of variance (ANOVA). Significant main and interaction effects were temperature ($p << 0.01$) and moisture level ($p = 0.013$). Figure 2 displays resulting microbial inactivation rates (i.e., each data point represents the average of 3 samples) and their dependence on temperature and moisture levels. In general as the temperature increased, the number of *E. coli* rapidly decreased. For each moisture level, the greatest inactivation rate occurred at the highest temperature, 38ºC. These results are consistent with the study by Price and Sowers (2004) [18]. In addition, each curve representing different temperatures within a fixed moisture level clearly demonstrated that microbial inactivation was temperature-dependent [19].

Performance of ANN

The k-fold cross validation method was used to select the best input parameter in the ANN trained with the backpropagation algorithm. A total of 9 subsets were chosen and compared for their individual generality and suitability as a representative input dataset. Predictive performances of backpropagation were evaluated with four different statistical measures: correlation coefficient (R), mean error (ME), mean square error (MSE) and the root mean square error (RMSE). The ANN responses are more precise if R, ME, MSE and RMSE values are found to be close to 1, 0, 0 and 0, respectively. Table 1 listed statistical criteria for comparison with predictive performance from 9 categorized subsets. Overall, categorized dataset 8 appeared to perform best among the 9 subsets in terms of all four performance criteria.

Figure 3 displays responses of trained networks to changes in governing factors (e.g., momentum, learning coefficient ratio, learning rate, transfer function and number of iterations). Momentum did not have a significant influence on simulation results (0.1 to 0.5 within a range of $R^2 = 0.850–0.853$, respectively).

A high learning rate resulted in increased accuracy for training and testing by way of changing the weight vector significantly from one cycle to another. However, a learning rate higher than one did not produce better results. Number of iterations was also considered one of the main influencing factors on model performance. Simulation results were varied but 50,000 iterations showed the best result.

Learning rate and momentum were initially set to be 0.15 and 0.4, respectively. At 10,000 training cycles, they were reduced to 0.075 and 0.2, respectively, and they were further reduced to 0.0187 and 0.05, respectively, at 30,000 training cycles. Figure 4 compares experimentally calculated (k_{d_Exp}) and ANN computed (k_{d_ANN}) microbial inactivation's depending upon different temperature and moisture conditions.

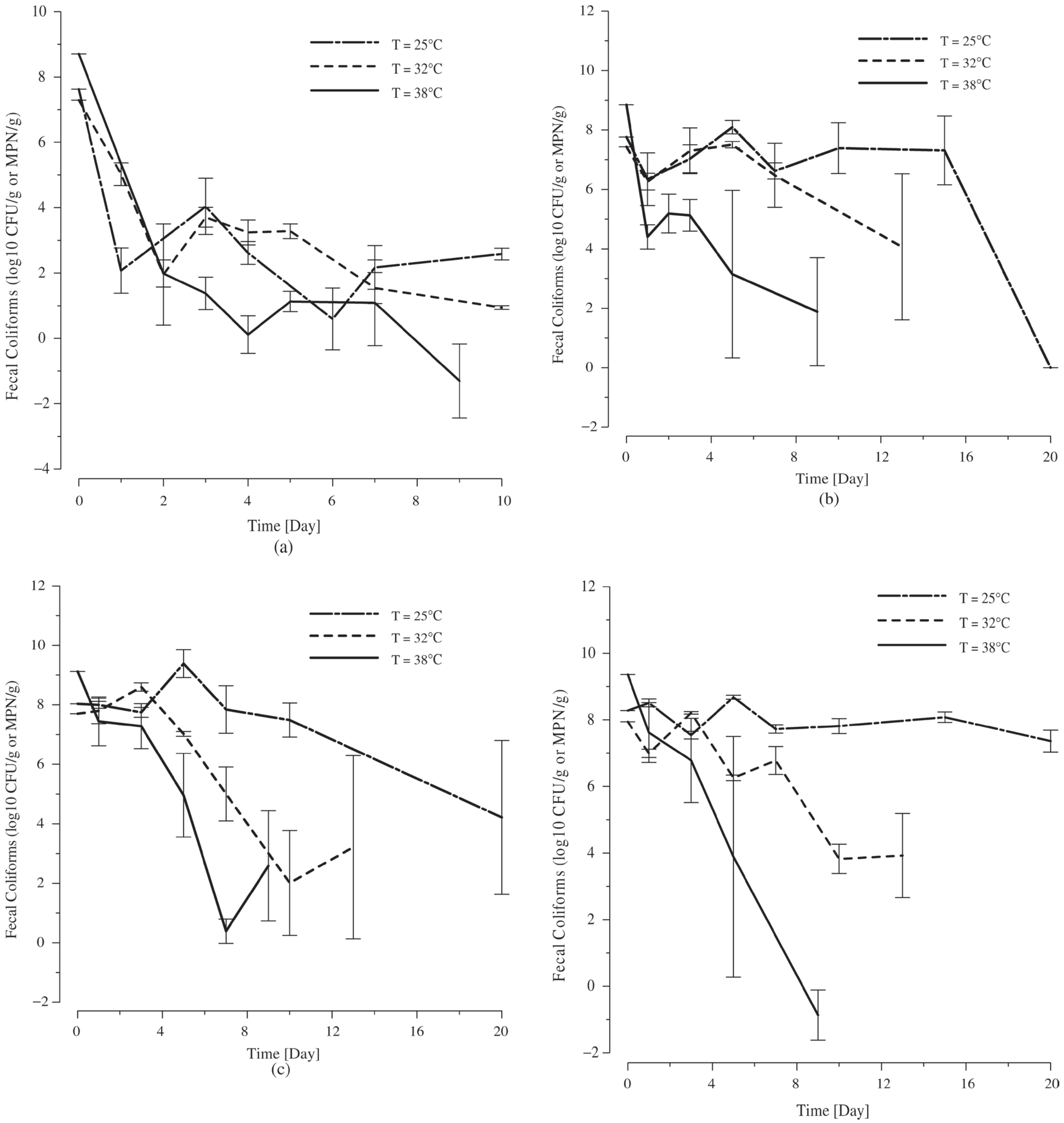
Overall, the predictive performance of ANN using backpropagation algorithm is displayed in Figure 5. Notice the R^2 value is 0.947 or 94.7%.

CONCLUSION AND DISUSSION

Survival curves for bacteria subjected to combined stress conditions are often complicated. This study selected temperature and moisture content as the main environmental factors affecting *E. coli* survival and conducted a series of experiments to study micro-

Table 1. Predictive Performance Efficiencies of Backpropagation to Different Input-Output Subsets (1–9).

Categorized Dataset	Performance			
	R	ME	MSE	RMSE
1	0.471	0.356	0.058	0.241
2	0.800	0.313	0.001	0.037
3	0.580	0.445	0.003	0.053
4	0.921	0.306	0.014	0.117
5	0.945	0.286	0.004	0.064
6	0.860	0.483	0.008	0.089
7	0.949	0.269	0.020	0.143
8	**0.977**	**0.209**	**0.001**	**0.025**
9	0.929	0.358	0.045	0.212



(a)

(b)

(c)

(d)

Figure 2. Survival curves for fecal coliform at moisture levels of (a) 10 %, (b)35 %, (c) 65 % and (d) 80% under three different temperatures (25, 32 and 38°C). Each point represents the average value of three replicates, and the error bars indicate one standard deviation from the mean.

bial inactivation for combinations of temperature and moisture treatment levels. The multilayer backpropagation algorithm developed in this study successfully estimated microbial inactivation rates by using partial data sets to train the neural network to represent the overall characteristics of the inactivation rate for fecal coliforms as a function of temperature, moisture content and the combination of the two factors. A resulting high correlation coefficient and low statistical error rates indicate that ANN modeling has potential to serve as a tool to predict fecal coliform survival in biosolids as a function of temperature and moisture levels.

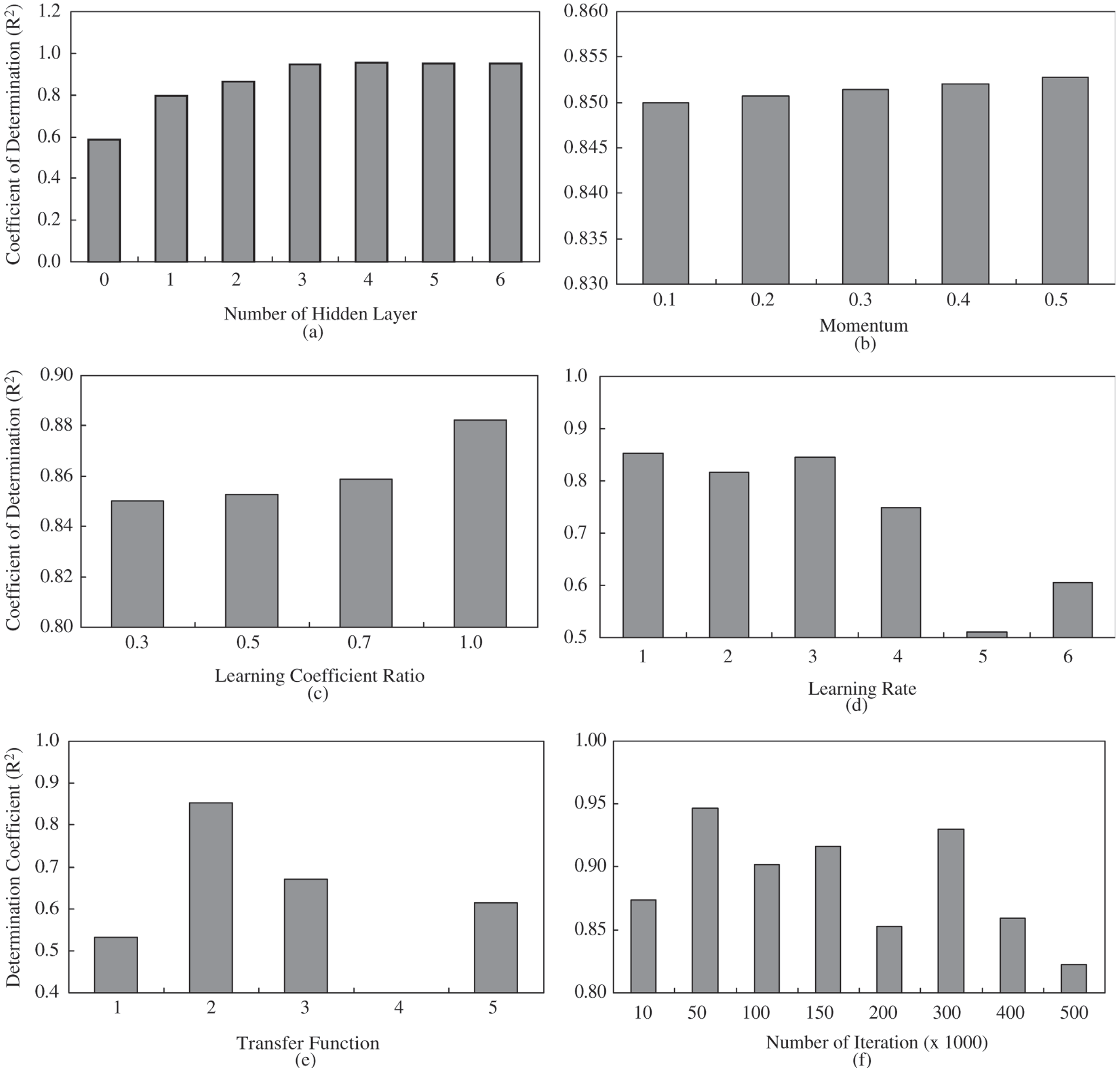


Figure 3. *Responses of Backpropagation algorithm results to changes of control parameters in a network. Regarding different bar graphs in this figure, (a) is for number of hidden layers; (b) for is momentum; (c) for is learning coefficient ratio; (d) is for learning rate; (e) is for transfer function and f for is number of iterations (×1000). Note: Learning rate (1 is Delta, 2 is Norm-Cum-Del, 3 is Ext DBD, 4 is Quick Pro, 5 is Max Pro and 6 is Delta-bar-delta) and Transfer function (1 is Linear, 2 is TanH, 3 is Sigmoid, 4 is DNNA, and 5 is Sine).*

The best use of these predictive capabilities may be for a public owned treatment plant to determine optimal timing for specific microbial testing required to designate Class A biosolids.

Results from this study give impetus for further investigation into specific pathogen level prediction of biosolids using ANN modeling. However, for ANN modeling to be an effective tool for predicting pathogen levels over time in biosolids at a full scale level, there are a number of caveats needing to be addressed. These include recognition of variations in sludge matrices due to differences in digestion, dewatering and drying methods (e.g., aerobic, anaerobic with the inclusion of polymers or not, heat-drying, air-drying and composting facilities). They also include spatial heterogeneity of microbial levels in sludge especially in aerobically digested matrices, temporal variability due to inconsistent disposal rates, explicit responses of

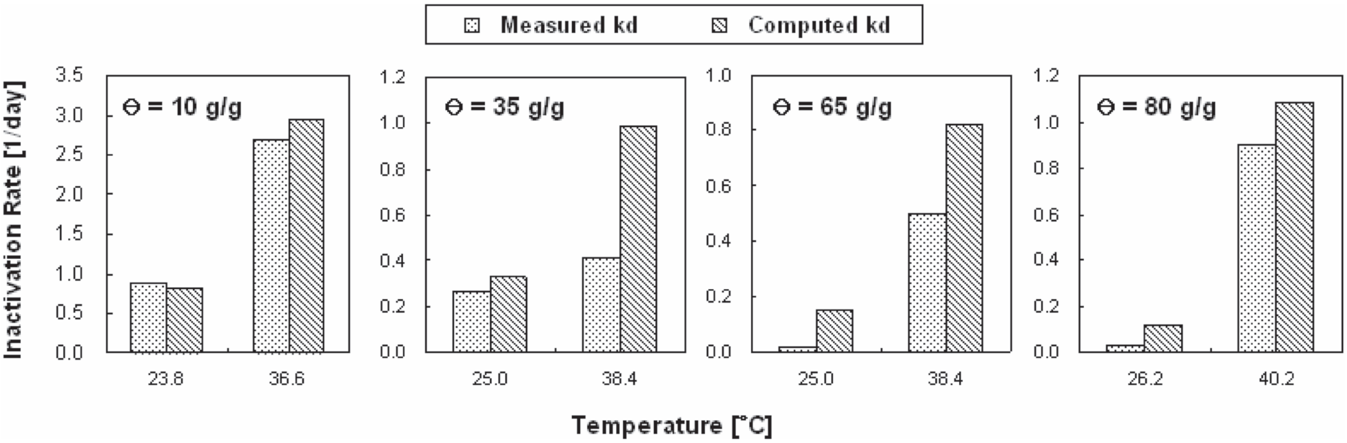


Figure 4. *Comparison of computed and measured inactivation rates for each moisture level.*

specific pathogens to temperature, moisture and environmental factors, consistency of drying methods such as frequency of biosolids turning in drying beds and environmental variability (e.g., rainfall, wind, ambient air temperature and incident solar radiation).

Methods used to overcome these issues include ANN modeling based on various inputs such as sludge matrices, microbial assays of specific pathogens (i.e. *Salmonella sp.*, enteric virus and helminth ova), environmental parameters (e.g., RH, rainfall, air temperature and incident solar radiation—all of which impact moisture evaporation) and quantifiable biosolids management practices that can impact spatial or temporal variability. Use of ANN models as a decision support tool for microbial testing can be an invaluable method for validating best management practices for biosolids production and optimizing timing for biosolids microbial quality testing. This results in time and monetary savings.

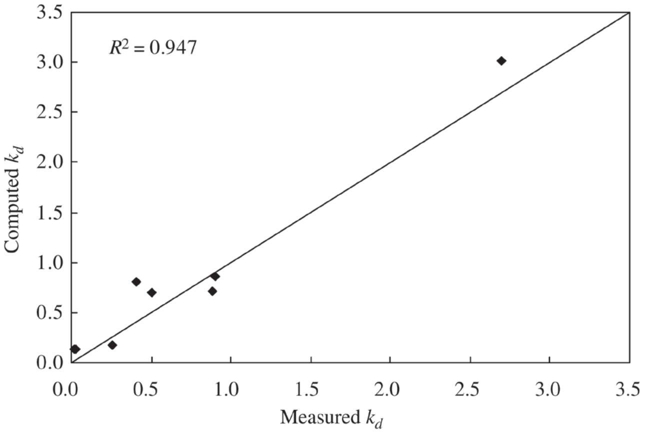


Figure 5. *Overall performance of Backpropagation to predict microbial inactivation rate (k_d) depending upon variation of temperature and moisture levels.*

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Natural Zeolite Reduces Salinity and Heavy Metal Availability of Compost Produced from Sewage Sludge-rose Residue Mixture

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ABSTRACT: Despite positive effect from compost on soil physical, chemical, and biological properties, its farm application may potentially increase salinity and heavy metals' content in soils. The aim of the study was to investigate effect of natural zeolite as an ingredient in reducing salinity and heavy metals' availability in compost. Four rates of natural zeolite were added to the sewage sludge-rose residue mixture and thoroughly mixed. Salinity level in the final compost produced was decreased while increasing the amount of natural zeolite used. Addition of 5, 10, and 15% zeolite resulted in 11.1, 25.9, and 42.6% reduction of salinity level. Addition of zeolite proportional to the applied rate significantly decreased zinc, iron, copper, and cadmium concentrations in the produced compost. Results suggest addition of zeolite to the sewage sludge-rose residue mixture could effectively improve applicability of the end product from the composting process on agricultural lands by reducing its salinity and heavy metals availability.

1. INTRODUCTION

COMPOSTING is a natural biological decomposition process that can produce a stable and useable end-product from potentially hazardous organic wastes from domestic, agricultural, and industrial origin [30]. Compost can increase soil organic matter content, improve tilth and water-holding capacity, suppress weeds, and provide a long-term supply of nutrients as organic material decomposes [11,21]. For these reasons, composting has been advocated as one component of sustainable agriculture [3, 10]. The quality of compost before its land application has to be well understood. Quality of compost is related to different factors such as stability and maturity [14, 29], pH, and electrical conductivity (EC) [4,28]. Salinity is particularly an important factor in determining applicability of compost for agricultural lands. Elevated salinity of compost often makes it difficult to use as an amendment in agricultural soils or as a growing media [4, 28].

Several studies demonstrated elevated concentrations of soluble salts in end products from the composting process. Ticoya (2000) showed that salinity of compost increased from 2.83 dS m^{-1} to 4.40 dS m^{-1} after 77 days of incubation [26]. In that experiment as the process progressed, soluble salts' concentration increased which reflected the progressive mineralization of the organic matter. Soliva (1999) reported relative concentrations of all initial components were increased during the composting process particularly in the final product from composting. Increase of EC of cattle manure after composting has been attributed to increases in microbial activity [25]. In contrast, some research studies demonstrated salinity of compost was reduced during the composting process due to the NH_3 volatilization or salt leaching [1].

High concentration of heavy metals is another potential hazard associated to the compost particularly when biosolids is used as a component of the compost. Therefore, it is necessary to reduce salinity and heavy metals' content in compost before its application to agricultural lands.

It has been reported that addition of sawdust and recycled materials during the composting process may reduce salinity of the end product [1]. Natural zeolites have also reported to be effective in removal of Pb, Cd, Zn, Mn, and NH_4^+ from effluents [32]. Zeolites are crystalline hydrated aluminosilicate of alkali and alkaline earth cations possessing an infinite and open three-dimensional structure [20]. The microporous crystalline structure of zeolites is able to adsorb spe-

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cies having diameters that fit through surface entry channels, while larger species are excluded, giving rise to molecular sieving properties that are exploited in a wide range of commercial applications [14, 18].

Several farmers in central Iran produce compost from a mixture of greenhouse-grown rose flower residue and sewage sludge and use it as soil amendment or growing media. Compost produced supplies a high content of organic matter with favorable effects on soil properties. High salinity and heavy metals' content (e.g., Cd, Fe, Zn, and Cu) are major limiting factors regarding using the produced compost in soil. The objective of the present study was to investigate possible use of zeolite as an amendment to reduce potential hazards of compost and thus to improve its applicability in soil.

MATERIAL AND METHODS

Sample Preparation

Natural zeolite was taken from the Afrand Tooska Company in Iran. The result of X-ray diffraction (XRD) analysis displayed that the used zeolite is mainly clinoptilolite micronizehe.

Clinoptilolite is a hydrated alumosilicate of alkaline and alkaline earth metals (Na, K, Ca, and Mg). Therefore, the ideal chemical formula for this mineral may be written as:

$$M\ x/n\ [Al_xSi_yO_2(x+\ y)\ pH_2O]$$

where M is the monovalent (Na, K) or divalent (Ca^{2+}, Mg^{2+}, and Ba^{2+}) cation and n is the ion valence [8]. Chemical composition and main characteristics of the used zeolite are presented in Table 1.

The rose residue inclusive stems and leaves were collected from the commercial greenhouse unit of the Soilless Culture Research Centre, Isfahan University of Technology, Iran. Sewage sludge was taken from Shahinshahr refinery located in north Isfahan. The sludge used in this study was secondary anaerobically digested municipal sewage sludge from the city of Isfahan. Characteristics of the sewage sludge are displayed in Table 2.

This study was conducted in the research greenhouse of the university with 25/18°C temperature and 80% relative humidity. Initial material was put in closed-ended and drainable plastic boxes. Each box had a volume of 40 L. Sludge and rose residue were mixed at a 2:1 ratio (w/w). Then, zeolite was added to the mixture at four levels including 0, 5, 10, and 15% by volume. During composting the mixture was aerated daily. Temperature was measured daily at the center of the mixture, but the maximum temperature was reported weekly [27]. Maximum temperatures for the composting process for 0, 5, 10 and 15 percent of zeolite reached 40, 48, 54, and 50°C, respectively.

Chemical Analyses

Representative compost sub-samples were taken once a week during composting and analysed for electrical conductivity, pH, and available K, Mn, Na, Zn, Fe, Cu, and Cd contents. The pH and EC were respectively measured using a digital pH-meter (Model Ohm-620, Metrohm AG Herisau Switzerland) and a digital EC-meter (Model Ohm-644, Metrohm AG Herisau Switzerland) in water extract obtained by mechanically shaking samples with distilled water at a solid:water ratio of 1:5 (w/v) for 1 h [13].

To measure concentration of mineral elements, 1 g of each sub-sample of compost was placed in an oven at 70°C until reaching a constant weight. Dry samples were combusted at 550°C for 8 h and mineral elements in the ash were extracted using HCl 2 M solution [7]. Concentrations of Mn, Fe, Zn, Cu, and Cd in the digest solutions were determined by atomic absorbtion spectrophotometer (AAS) (Perkin Elmer 3030, USA).

Table 1. Chemical Composition of Natural Clinoptilolite used in this Experiment.

Element	Amount (wt%)
SiO_2	66.5
Al_2O_3	11.8
Fe_2O_3	1.30
CaO	3.11
MgO	0.72
Na_2O	2.01
K_2O	3.12
P_2O_5	0.01
MnO	0.04
TiO_2	0.21

Table 2. Characteristics of Sewage Sludge used for Making Compost.

Characteristic	Amount
TS (%)	6.8
EC (dS m^{-1})	5.84
pH	6.5
P (%)	0.62
K (%)	0.25
Fe (mg kg^{-1})	11,910
Zn (mg kg^{-1})	710
Cu (mg kg^{-1})	450
Cd (mg kg^{-1})	6

Concentration of K was measured by flame photometer (Model410, corning, England), respectively.

Statistical Analysis

The experiment was set up in a completely randomized design with three replicates. All data were subjected to analysis of variance and means were compared using least significant differences (LSD) when the F test indicated significance at $P < 0.05$ [23].

RESULTS AND DISCUSSION

EC and pH

The pH value of the mixture of sewage sludge and rose residue was 7.8 ± 0.3 before composting. The pH value was reduced at the end of first week of the composting and thereafter, remained unchanged or slightly increased. Please see Figure 1.

Changes in pH have been considered as a possible indicator of biological activity related to organic matter degradation. Some authors have demonstrated that pH of certain materials decreases during composting [6]. Reduced pH due to composting is partly attributed to acid formation during decomposition of organic matter [31]. Oxic and anoxic conditions at the first step of composting and produced by acetic acid and ammonia effect degradation rate of organic materials [15, 26].

Table 3. EC and pH Values of the End-product from Composting Based on Zeolite Application Rate.

Zeolite rate (%)	EC (dS m^{-1})	pH
0	6.23^a	7.9^a
5	5.45^b	7.7^a
10	4.54^c	6.9^b
15	4.02^d	6.3^b

Values with the same letter within each column are not significantly different at $P < 0.05$.

Composts produced from the zeolite-amended materials had significantly ($P < 0.05$) lower pH compared with those produced from the no zeolite-amended materials. Please see Table 3.

Addition of natural zeolites to the initial materials' mixture resulted in a significant increase of porosity in the composting mixture and stimulated microbial activity and biodegradability of organic matter. Similar to our results, Turan et al. (2009) reported that pH values from compost decreased when natural zeolites were added [27]. Reduction in the compost pH value may be related to increased nitrification that may take place during the curing stage [12].

Lower pH during the composting process is positive and causes reducing ammonia and other odors' to release. Ammonia volatilization from poultry litter dramatically increases once pH rises above 7.0 [12].

Initial EC value of the sewage sludge mixture and rose residue was 5.60 ± 0.10 dS m^{-1}. It significantly increased at the end of the first week of composting and thereafter remained unchanged or slightly decreased. Please see Figure 2.

Turan, et al, (2009) reported that during the initial

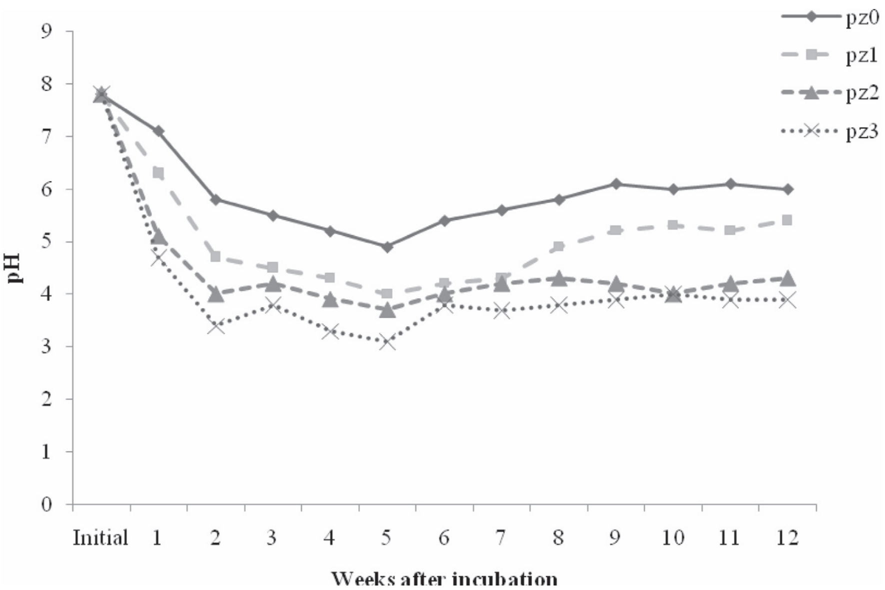

Figure 1. Changes in the pH of the sludge-rose residue mixture during composting process as affected by addition of different zeolite rates. In the legend pz0, pz1, pz2, and pz3 represent application of 0, 5, 10, and 15% zeolite, respectively.

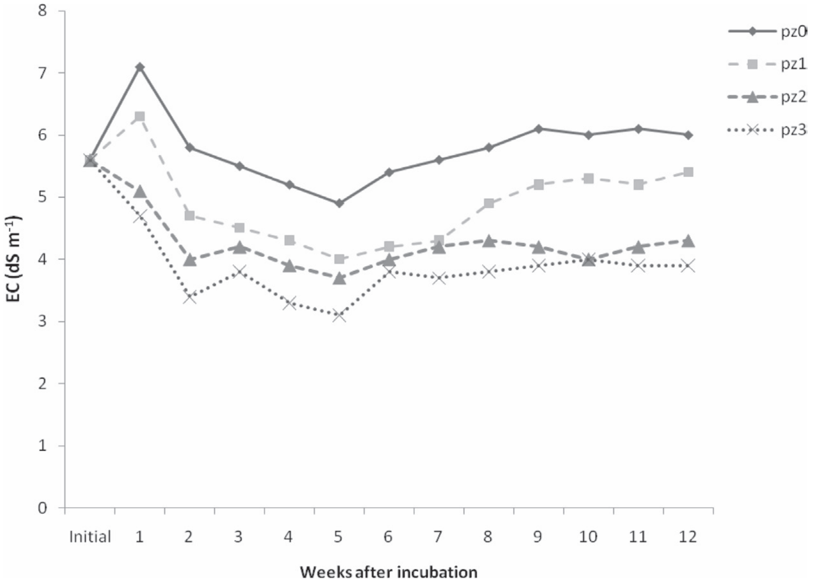

Figure 2. *Changes in the EC of the sludge-rose residue mixture during composting with addition of different zeolite rates: pz0, pz1, pz2, and pz3 represent application of 0, 5, 10, and 15% zeolite, respectively.*

phase of composting, electrical conductivity of the composting mixture is reduced due to the high concentrations of organic acids. Relatively soon and afterwards, the acids are decomposed and electrical conductivity is increased [27]. Elevated EC for the mixture after the first week of the composting is partly due to organic matter mineralization and release of inorganic soluble salts [6]. Concentration of soluble salts in the composting mixture reflects progressive mineralization of the organic matter [6]. Elevated EC of cattle manure after composting has been attributed to increased microbial activity [22]. In the thermophilic stage, a high amount of nutrients is consumed. Microorganisms ingest these nutrients, which is why these nutrients are not soluble in water and salinity is low. But as decomposition progresses, the microorganisms do not consume as many nutrients so the nutrients start to accumulate in the soluble phase [22]. The EC of the compost was decreased significantly in the presence of added zeolite (Table 3).

Addition of 5, 10, and 15% zeolite resulted in a decrease of the compost EC by 11.1, 25.9, and 42.6%, respectively. Similar to our findings, Turan et al (2009) reported that salinity level in the end-produced compost decreased with increasing amount of zeolite used. Natural zeolite carries a negative charge balanced by freely moving cations with positive charges. It has a very open framework with a network of pores giving it a large surface area for trapping and exchanging different cations [27].

The ideal value of EC for compost depends on different factors (i.e., type of compost, end use, climate, and plant type) [5]. Considering soil conditions in central Iran and standards presented by the Soil and Water Research Institute [24] as well as according to Australia's new compost standards [5], ideal compost for use in agricultural soils of Iran should have an EC no higher than 10 dS m^{-1} in aqueous extract (1:5 w/v). Therefore, composts produced in the presence of 10 and 15% zeolite levels have salinity levels suitable for use in agricultural soils of Iran.

Table 4. Concentration (%) of Mineral Elements in Compost as Affected by Different Zeolite Application Rates.

Element	Initial mixture	End-product Zeolite rate (%) 0	5	10	15
Ca (%)	8.5	8.3	5.8	4.2	3.3
Mg (%)	0.90	0.70	0.52	3.1	0.23
K (mg kg^{-1})	120	100	67	54	40
Na (mg kg^{-1})	80,000	80,000	58,000	49,000	39,800
Fe (mg kg^{-1})	12,100	11,600	7,100	5,600	4,400
Zn (mg kg^{-1})	780	670	400	250	200
Cd (mg kg^{-1})	6.0	5.1	4.2	2.0	<0.02
Cu (mg kg^{-1})	450	410	260	80	50

Concentration of Elements in the Compost

Addition of zeolite proportional to the applied rate decreased concentration of Zn, Fe, Cu, Cd and K in the composting end-product. See Table 4.

Reduction of metal concentration of the composting end-product in the presence of 5, 10, and 15% zeolite was 30, 67, and 100% for Cd. It was 43, 82, and 89% for Cu. It was 49, 68, and 75% for Zn. Finally, it was 42, 54, and 64% for Fe (Table 4). Accordingly, Zorapas et al. (2000) reported clinoptilolite has an ability to readily take up almost all metals that are bound to exchangeable and carbonate fractions. Zeolite adsorbs all metals bound in exchangeable and carbonate fractions according to the following selectivity series: Cu > Cr >Fe > Ni > Mn > Pb > Zn [32]. Another researcher also reported the microporous crystalline structure of zeolites is able to adsorb species that have diameters that fit through surface entry channels while larger species are excluded giving rise to molecular sieving properties that are exploited in a wide range of commercial applications [2, 18]. Natural zeolites have a particularly effective capability for removal of Pb, Cd, Zn, and Mn from effluents [32].

CONCLUSION

A major limitation regarding farm application of compost results from its high heavy metal content and salinity. The study demonstrated that concentration of different heavy metals, EC, and pH of the composting end-product was decreased with the addition of natural zeolite (clinoptilolite) as an amendment ingredient. According to results, addition of zeolite to the mixture of sewage sludge and rose residue could effectively improve properties of the compost produced and enhance its applicability for use in agricultural lands or as a growing media for horticulture, gardening, and landscaping.

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Cadmium Phytoextraction Efficiency by *Sorghum bicolor* and *Helianthus annuus*

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ABSTRACT: The objective of this study was to determine Cd phytoextraction efficiency for *Sorghum bicolor* and *Helianthus annuus*. Pot experiments were carried out in a greenhouse using Cd contaminated soils at 0 and 5 mg Cd kg^{-1}. Biomass and Cd concentrations were measured in shoots and roots of the plants. Sequential extraction was used in contaminated soil before and after plants cultivation. Results demonstrated that *Helianthus annuus* had a higher shoot biomass production compared to *Sorghum bicolor.* Accumulation of Cd in shoots of *Helianthus annuus* was greater than that of *Sorghum bicolor.* Values of bioconcentration factor (BCF), translocation factor (TF) and phytoextraction efficiency (PR) indicated *Helianthus annuus* had a higher potential to translocate and move Cd from the plant roots to the shoots than *Sorghum bicolor.* Based on sequential extraction results, phytoextraction of Cd by *Sorghum bicolor* and *Helianthus annuus* decreased the percentage of exchangeable and bound to carbonates forms of Cd in the soil.

1. INTRODUCTION

CADMIUM contamination of agricultural soils is an important concern [6, 32]. Cadmium contamination of agricultural soils may happened by the application of sewage sludge, urban composts, pesticides, fertilizers, emissions from waste incinerators, waste water irrigation, and residues from metalliferous mining and the metal smelting industry [14, 33]. Cadmium is a heavy metal that is highly toxic to plants, animals, and humans. This is especially important when contaminated lands are used for crop cultivation, since Cd is easily transferred from soils into the food chain [5, 8].

Many technologies have been developed to remediate heavy metal contaminated soils. Phytoextraction is an economical alternative remediation method, in which plants are used to extract metals from soils. Selection of metal tolerant plants to efficiently remove metals is the first step. Phytoextraction efficiency is determined by two key factors: metal hyperaccumulating capacity and biomass production [15]. Many studies have indicated that certain varieties of high-biomass crops displayed heavy metal tolerance, such as Indian mustard (*Brassica juncea*), oat (*Avena sativa*), maize (*Zea mays*), barley (*Hordeum vulgare*), sunflower (*Helianthus annuus*), and ryegrass (*Lolium perenne*) [9,17,25,28]. Biomass can express the tolerance of plants to toxic metals indirectly. However, most metal hyperaccumulators are small and slow growing [10].

In case of Cd, only a few field trails have been conducted to determine phytoextraction potential for Cd-accumulating plants [12, 13,19,37]. Most of the field studies have shown that it would take approximately 25–500 yr to reduce the concentration of Cd in contaminated soils below a critical limit using Cd-hyperaccumulators such as *Thlaspi caerulescens*, *Sedum aflredii*, or *Viola baoshanensis* [12,27,37] or high-biomass Cd-accumulators such as *Salix* species, *Brassica juncea*, *Helianthus annuus*, or *Zea mays* [13,19]. Screening of new species/cultivar showing a higher Cd removal capacity and optimizing agronomic practice are the two main approaches for further improvement of Cd phytoextraction potential [4,16,31].

The objectives of this study were to 1) compare Cd phytoextraction efficiency of *Sorghum bicolor* and *Helianthus annuus*, 2) determine concentration of Cd in plant parts during crop cycle and 3) evaluate Cd uptake and translocation capacity in *sorghum bicolor* and *Helianthus annuus*, and 4) determine different forms of cadmium in contaminated soil.

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MATERIALS AND METHODS

Greenhouse Experiment

Pot experiments were conducted in a randomized complete block design with three replication at the greenhouse of Islamic Azad University, Khorasgan branch. Two annual high-biomass yield crops, sorghum (*Sorghum bicolor*) and sunflower (*Helianthus annuus*) were used to determine their Cd phytoextraction potentials. Soil samples were air-dried, homogenized, and sieved (2 mm). Soil was contaminated by Cd ($CdCl_2$) at 5 mg Cd kg^{-1}. Soil samples were fertilized with 100 mg kg^{-1} N and K ($(NH_4)_2SO_4$ and K_2SO_4) respectively and transferred to plastic pots (27 cm height × 25 cm diameter).

Three seeds from each of the plants were sown into pots and were irrigated with deionized water at field capacity.

Plant and Soil Analysis

Soil chemical and physical properties are displayed in Table 1. Plants were harvested after 75 days, washed thoroughly with deionized water, and then divided into roots and shoots and oven-dried for 48 hours at 75°C. Soil pH was measured in suspension using a ratio of 1:2 (*W*/*V*) soil and deionized water. Plant and soil samples were digested using a mixture of acid [HNO_3+$HClO_4$ (3:1, v/v)] [24]. Plant available Cd in soils was extracted with diethylene-tetramine-pentaacetate acid (DTPA). Cd concentrations in the plant and soil samples were determined by flame atomic absorption (FAAS, PerkinElmer, 3030) [2].

Table 1. Soil Chemical and Physical Properties.

Soil Characteristic	Value
pH (1:2 H_2O w/v)	8.2
EC (dS m^{-1})	2.5
CEC (meq 100g^{-1})	16
OM (%)	2
N (%)	0.2
P (mg L^{-1})	70.8
K (mg kg^{-1})	246
Na (meq L^{-1})	13.1
Ca (meq L^{-1})	7.6
Mg (meq L^{-1})	2.4
Total Cd (mg kg^{-1})	5
DTPA- Extractable Cd (mg kg^{-1})	3.3
Sand (%)	15
Silt (%)	48.4
Clay (%)	36.6
Soil texture	Silty Clay Loam

Phytoextraction Efficiency

Phytoextraction efficiency of plants was calculated as follows [18, 35]:

$$PR = (C_{plant} \times M_{plant}/C_{soil} \times M_{rooted\ zone}) \times 100$$

where M_{plant} is the mass of the harvestable above ground biomass produced in one harvest, C_{plant} is the metal concentration in the harvested component of the plant biomass, $M_{rooted\ zone}$ is the mass of the soil volume rooted by the species under study, and C_{soil} is the metal concentration in the soil volume.

The translocation factor (TF) of Cd from roots to shoots and bioconcentration factor (BCF) was calculated as follows [3,20] :

$$TF = [Cd]_{shoot}/[Cd]_{root}$$

$$BCF = [Cd]_{shoot\ or\ root}/[Cd]_{soil}$$

Sequential Extraction of Cd

The sequential extraction procedure of Cd suggested by Rauret et al. [23] was used in this study. The acid-soluble phase (Step 1) is made up of exchangeable Cd bound to carbonates. It is the most labile of the fraction. The reducible phase (Step 2) consists of metals bound to iron and manganese oxides. The oxidisable phase (Step 3) represents the amount of metal bound to organic matter and sulphides. The residual Cd content (fraction 4) was determined by digestion with aqua regia. This phase is Cd that is associated with crystalline structure and minerals. See Table 1.

Statistical Analysis

Statistical analyses were performed using SPSS 16 software and comparison of means was done with Duncan test at 5% level.

RESULTS

Plant

Biomass Production

The root dry weight biomass of *S.bicolor* and *H.annuus* in uncontaminated and contaminated soils are displayed in Figure 1(a) and in Figure1(b). Re-

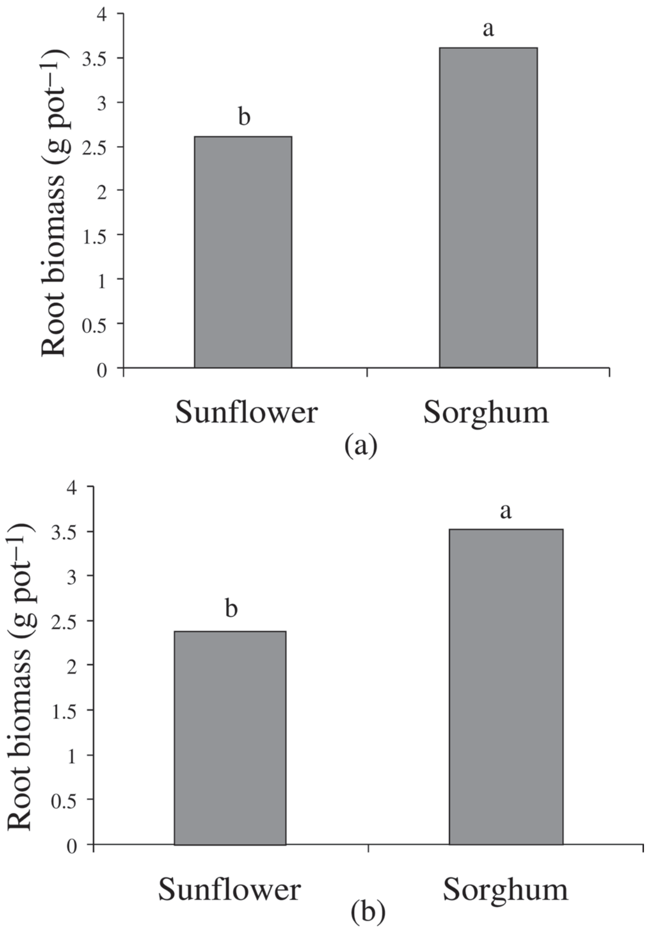

Figure 1. *The root dry weight biomass production (g pot^{-1}) of sorghum and sunflower grown in uncontaminated (a) and contaminated (b) soils.*

garding both, soils root dry weight of sorghum was significantly ($p < 0.05$) greater than that of the sunflower. Average root biomass production (Dry weight) in uncontaminated soils were 3.6 and 2.6 g pot^{-1} for S.bicolor and H.annuus respectively [Figure 1(a)]. The root dry biomass of plants in contaminated soil were 3.5 and 2.4 g pot^{-1} for *S.bicolor* and *H.annuus* respectively [Figure 1(b)].

As shown in Figure 2(a) and in Figure 2(b) shoot dry weight biomass for the sunflower was more than for the sorghum. There was a significant difference between shoot dry weight production from two plant species in contaminated and uncontaminated soils ($p < 0.05$). Shoot dry weight biomass production in uncontaminated soils were 29 and 19.6 g pot^{-1} for *H.annuus* and *S.bicolor* respectively [Figure 2(a)]. The shoot dry weight biomasses of plants in contaminated soils were 21 and 14 g pot^{-1} for *H.annuus* and *S.bicolor* respectively [Figure 2(b)]. Results display that Cd contamination of soil causes a reduction of yield for both plants.

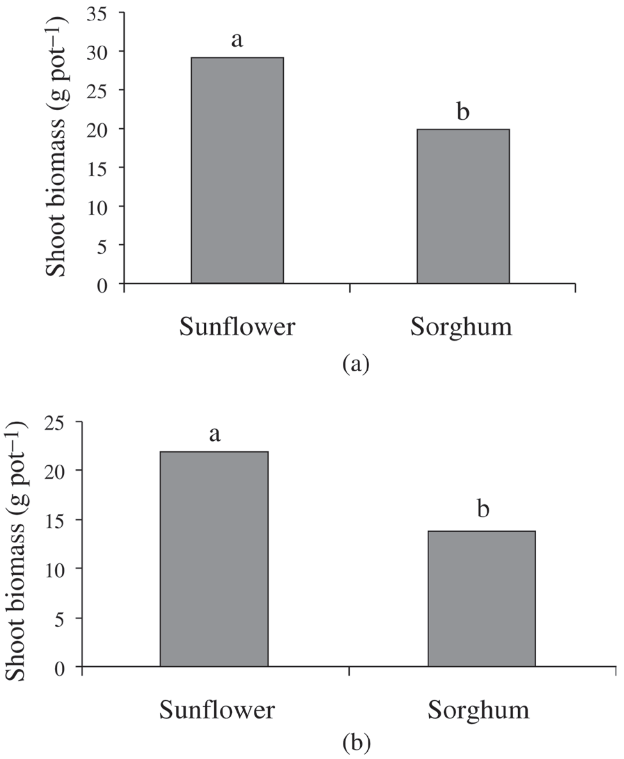

Figure 2. *Shoot dry weight biomass production (g pot^{-1}) of sorghum and sunflower grown in uncontaminated (a) and contaminated (b) soils.*

Cd Accumulation in Plant Tissues

In uncontaminated soil, Cd concentrations in roots and shoots of both plants were below detection limits for the atomic absorption spectrophotometry method. Cadmium concentrations in roots and shoots of plants cultivated in contaminated soil are displayed in Figure 3(a) and in Figure 3(b). In contaminated soil Cd concentration in roots of sorghum was significantly ($p < 0.05$) higher than for sunflower [Figure 3(a)] while Cd concentration in sunflower shoots was significantly ($p < 0.05$) higher than sorghum [Figure 3(b)].

Bioconcentration and Translocation Factors

Table 2 displays the bioconcentration factors (BCF) and the translocation factors (TF) for the two plant species. The BCF value in shoots of sunflower was significantly ($p < 0.05$) more than for sorghum. The BCF value in roots of sorghum was more than in sunflower but this difference was not significant. There was a significant ($p < 0.05$) difference between total weight BCF of plant species. The total BCF value in contaminated soil was 9.8 and 4.8 for sunflower and sorghum, respectively. The sunflower was more efficient for bioaccumulating Cd than sorghum.

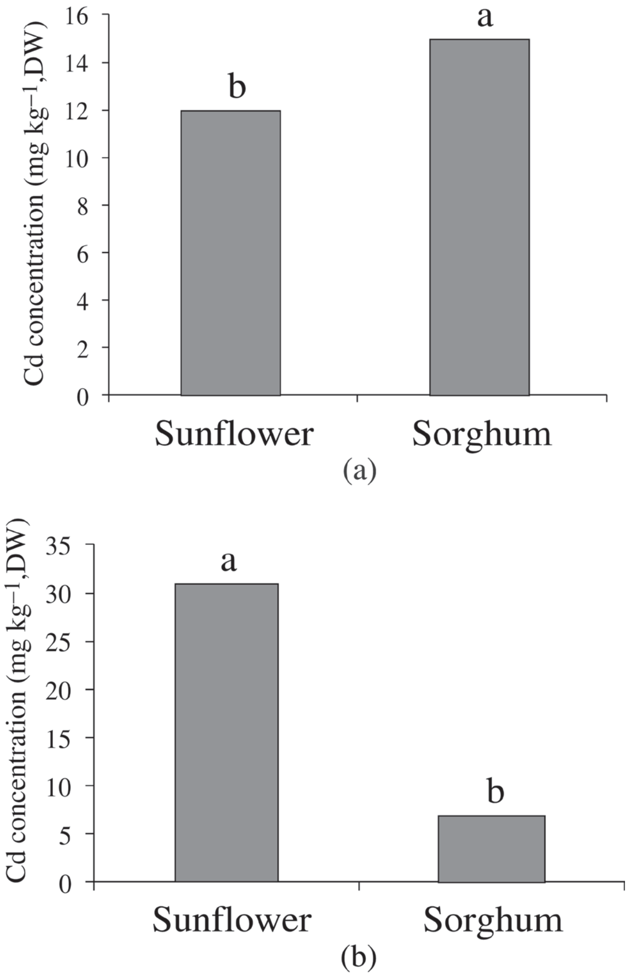

Figure 3. *Cd concentration in roots (a) and shoots (b) of sorghum and sunflower grown in contaminated soils.*

The TF value in sunflower was significantly ($p < 0.05$) more than for sorghum (2.5 and 0.43 respectively) and thus sunflower had higher potential to move Cd from roots to shoots than sorghum.

Phytoextraction Rate (PR)

Phytoextraction rates of Cd for sunflower and sorghum are displayed in Table 2. There was a significant difference between %PR of sunflower and sorghum that was cultivated in contaminated soil ($p < 0.05$). The %PR value was 2.1% and 0.28% for sunflower and sorghum respectively. In general sunflower had the higher %PR than sorghum.

Soil

Different Forms of Cd in the Soil

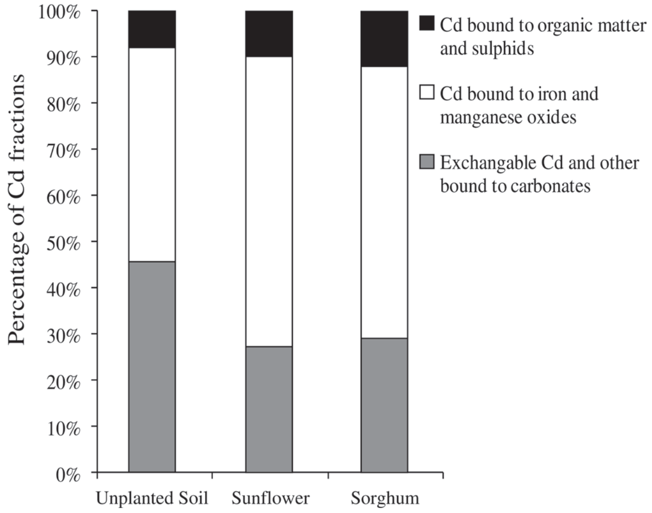

Figure 4. *Fraction of Cd in unplanted and planted soil.*

Figure 4 displayed Cd percentage in different fractions in contaminated soil before and after plants cultivation. The percentage of exchangeable and bound to carbonates forms of Cd decreased in the planted soil after 75 days. The percentage of bound to iron and manganese oxides form of Cd increased in the planted soil as compared with unplanted soil. There was no significant difference in percentage of bound to organic matter and sulphids and residual forms of Cd between planted and unplanted soils.

Data with different letters in the same column indicate a significantly difference at $p < 0.05$ based on Duncan test.

DISCUSSION

This study was conducted to determine natural ability of *H.annuus* and *S.bicolor* for phytoextraction of Cd from contaminated soils.

Phytoextraction efficiency depends on shoot biomass and the ability of a metal to be translocated to the shoots [35]. Biomass can express the tolerance of plants to toxic metals. High biomass plant species were better suited for phytoremediation of metal-contaminated soils [22].

It has been shown that high concentrations of heavy metals leads to a decrease in plant growth, and in this experiment, similar results were also obtained. As displayed in Figure 1(a), Figure 1(b), Figure 2(a), and Figure 2(b), roots and shoots biomass for plants cultivated in contaminated soil were less than that for uncontaminated soil. Madejon et al (2003) reported the amount of biomass in shoots and roots of sunflower in contaminated soil was less than for uncontaminated soil [11].

Table 2. Bio-concentration Factors (BCF), Translocation Factors (TF), and Phytoextraction Rates (PR) of Cd in Plants.

	BCF				
Cd (mg kg^{-1})	**Root**	**Shoot**	**Total plant**	**TF**	**%PR**
Helianthus annuus					
0	0 b	0 b	0 c	0 b	0 b
5	2.8 ± 0.17 a	7 ± 1.02 a	9.8 ± 0.93 a	2.5 ± 0.47 a	2.1 ± 0.41 a
Sorghum bicolor					
0	0 b	0 b	0 c	0 b	0 b
5	3.4 ± 0.25 a	1.4 ± 0.12 b	4.8 ± 0.03 b	0.43 ± 0.06 b	0.28 ± 0.01b

Data with different letters in the same column indicate a significant difference at $p < 0.05$ based on Duncan test.

Abou-Shanab et al., (2007) demonstrated that increase in concentration of heavy metals in soil leads to a decrease of biomass for the roots and shoots in *Zea mays, Sorghum bicolor, Helianthus annuus, Cynodon dactylon,* and *Conyza discordies* [1].

Results displayed that root biomass of *S.bicolor* was higher than for *H.annuus* [Figure 1(b)] while shoot biomass of *H.annuus* was more than *S.bicolor* in contaminated soil [Figure 2(b)]. Also, Cd concentration in roots of *S.bicolor* was more than *H.annuus* while Cd concentration in the shoots of *S.bicolor* was less than *H.annuus* in contaminated soil [Figures 3(a) and 3(b)].

Researchers have showed that *Helianthus annuus* is characterized by a high content of heavy metals in biomass and good remediation capacity due to high biomass production [26]. In addition, experiments have demonstrated that *Helianthus annuus* was found as a good accumulator in soil with a Cd concentration of 90 mg kg^{-1}[29].

The BCF values for two plant species were more than one. A BCF value higher than one indicates higher concentration of the element in the plant than that in the soil [21, 36]. The BCF values for *H.annuus* were more than for *S.bicolor*. Therefore, *H.annuus* was more suitable for Cd bioconcentration than *S.bicolor*.

As displayed in Table 2, the TF value for *H.annuus* was more than 1. TF values can describe movement and distribution of heavy metals in plants [21]. TF values higher than one indicate higher concentrations of metal in shoots and are one of the factors which indicate suitability of a plant for use in phytoextraction [14]. Therefore, *S.bicolor* had a low potential to move Cd from roots to shoots, while *H.annuus* had a effective function to Cd-translocation from roots to shoots.

Phytoextraction rate represents the percentage of an element removed by plant (dry) above-ground biomass from total element content in the soil during one cropping season [30]. Accumulation of more than 1% of total heavy metal contents present in the soil into the plant dry above-ground biomass should be achieved for economically feasible use of phytoextraction [7]. The phytoextraction rates of Cd for tested plant species displayed (Table 2) that 2% of the soil Cd was removed by sunflower but only 0.28% of the Cd was removed by sorghum during 75 days. Therefore, *H.annuus* was more suitable for Cd- phytoextraction than *S.bicolor*.

The exchangeable and bound to carbonate forms of Cd decreased in the planted soil. It means that the exchangeable form of Cd was easily uptaken by plants [34]. We observed that the Cd percentage bounded to Fe and Mn oxides were higher than for others fractions. A possible explanation could be the exchangeable form of Cd in planted soil was the predominant species for Cd uptake by the plant. Also, plant root exudates and rhizosphere microorganisms accelerated the stability process of added Cd in soils which might make the exchangeable form transform into other relatively stable forms such as organic forms and might help reduce potential of harm of Cd to the soil and water environment [34].

CONCLUSION

A comparison of Cd phytoextraction of *Helianthus annuus* and *Sorghum bicolor* in a pot study demonstrated that H.annuus had higher shoot biomass and higher Cd concentration in shoots than did *S.bicolor.* Also, *H.annuus* had BCF (shoot and total), TF, and %PR higher than one. Therefore, *H.annuus* had a higher potential for Cd translocation and accumulation in shoots compared to *S.bicolor.* Also, its Cd-phytoextraction efficiency was more than that of *S.bicolor.*

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Wood Waste from Mazandaran Wood and the Paper Industry as a Low Cost Adsorbent for Removal of a Reactive Dye

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ABSTRACT: Mazandaran wood and paper industry (MWPI) generates large amounts of wood waste (WW) daily. The aim of this study is to apply WW from this factory as a low cost and non-conventional adsorbent for reactive blue 19 dye removal from aqueous solutions. For this purpose, effects from main parameters during the adsorption process include contact time, initial pH, adsorbent dose and initial dye concentration. Maximum dye removal efficiency was observed to be 93.79% at a pH of 2 and with 16 g/L of WW. Maximum value of Q for this waste was 30.92 mg-dye/g adsorbent. For the real textile wastewater containing RB 19, the dye removal efficiency was obtained at 73.84%. The adsorption process follows a Langmuir isotherm equation and the pseudo-second order kinetic model.

INTRODUCTION

SOME synthetic dyes from wastewater effluents are toxic and may be carcinogenic [1]. Many methods are reported for removal of dyes from effluents [2]. These technologies can be divided into three categories including biological, chemical and physical methods [3, 4]. The adsorption process is an efficient method for removing dyes from effluent due to its low initial cost, simplicity of design, ease of operation and insensitivity to toxic substances [5, 6].

Recent investigations have been proposed using non-conventional low-cost adsorbents including natural materials and waste materials from industry and agriculture for dye removal [3, 7]. Treated tendu waste [8], grapefruit peel [9], char from waste bamboo [10], coffee grounds [11] and sewage sludge [12] were used as novel adsorbents.

Generally, a lot of wastes such as solid wastes from the pulp and paper industry have little or no economic value and they are disposed of using different methods. For example, pulp and paper sludge is usually disposed of in open dumps or in landfills, recycled as compost or incinerated for energy recovery in the manufacturing process [13]. Because of high cost problems related to disposal methods, utilization of waste materials as new products for other applications rather than the aforementioned methods has been proposed. Soil improver and building material [13] or metal ion removal [14] for pulp and paper sludge has been reported.

MWPI is the largest paper manufacture in Iran which produces 100–120 tons of air-dried sludge as well as 150 tons of WW daily. Recently, all these solid wastes from the MWPI factory are mixed and composted. In the author's previous studies, performance of dried pulp and paper sludge for removal of reactive blue 19 (RB19) using the adsorption process was investigated [15].

The aim of this study is to evaluate potential of WW from a MWPI as a low cost adsorbent for the removal of RB19 dye. For this purpose, the effect of contact time, pH, adsorbent dose and initial dye concentration on the performance of the adsorption process were examined. In addition, isotherms and the kinetics of the adsorption process were evaluated. Finally, treatment of real textile wastewater under optimum conditions was examined.

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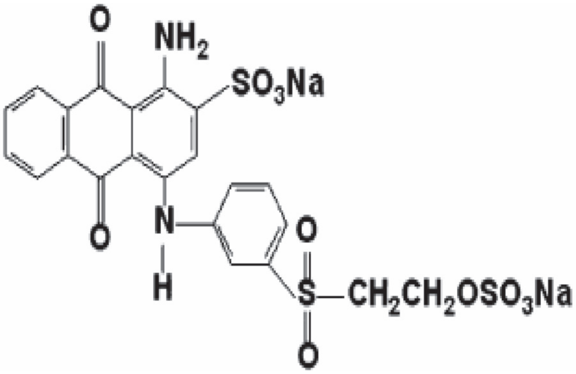


Figure 1. Chemical structure of RB19.

MATERIALS AND METHODS

Preparation of Synthetic Wastewater and Real Textile Wastewater

General properties of RB19 (provided by Alvan Sabet Company in Iran) are as follows: empirical formula = $C_{22}H_{16}N_2Na_2S_3$, molecular weight (MW) = 626.54, and λ_{max} = 594. Chemical structure of RB19 is presented in Figure 1. This dye is widely used in textile industries in Iran. For preparation of synthetic wastewater, a Stock dye solution of 1000 mg/L was prepared in double distilled water and then diluted to desired concentrations.

Also, real textile wastewater containing reactive blue 19 dye was collected from Negin-Nakhe-Beshel Company, in Mazandaran, North of Iran. This real wastewater has some additives including NaCl, Na_2CO_3, caustic NaOH, cationic ion exchange, detergent (ABS), cationic softener, antifoam and the other chemical agents. Characteristics of the real textile wastewater are as follows: RB19 concentration = 500 mg/L, pH = 10, chemical oxygen demand (COD) = 1000 mg/L and the nephelometric turbidity unit (NTU) = 7.94.

Preparation and Characterization of Adsorbent

WW used in this study was collected from MWPI. WWs from MWPI are derived from shell and putrefied wood from different types of trees such as beech, spruce, hornbeam, maple and oak. Collected WW was dried at room temperature for 24 hours in a laboratory. Materials were grounded and sieved using a 150 µm sieve. Particles less than the 150 µm sieve were then used as the adsorbent.

According to XRF analysis, the chemical composition of WW was 1.8% CaO, 0.45% K_2O, 0.33% MgO, 0.23% Al_2O_3, 0.16 % Fe_2O_3, 0.098% SO_3, 0.73% P_2O_5 and 0.046% SiO_2. The loss on ignition (LOI) which includes organic compounds from the WW was found to be 96.72% by weight. A scanning electron microscope (SEM) was also used to characterize the WW sample for morphological properties or characteristics. SEM pictures (Figure 2) display morphology of WW samples before and after the adsorption process. Textural properties of the adsorbent were assessed using N_2 adsorption/desorption isotherms at 77 K using an Autosorb 1 analyzer (Quantachrome Corporation, USA). Specific surface area (S_{BET}) was calculated by using the Brunauer–Emmett–Teller (BET) method. Textural characteristics of the adsorbent are provided in Table 1.

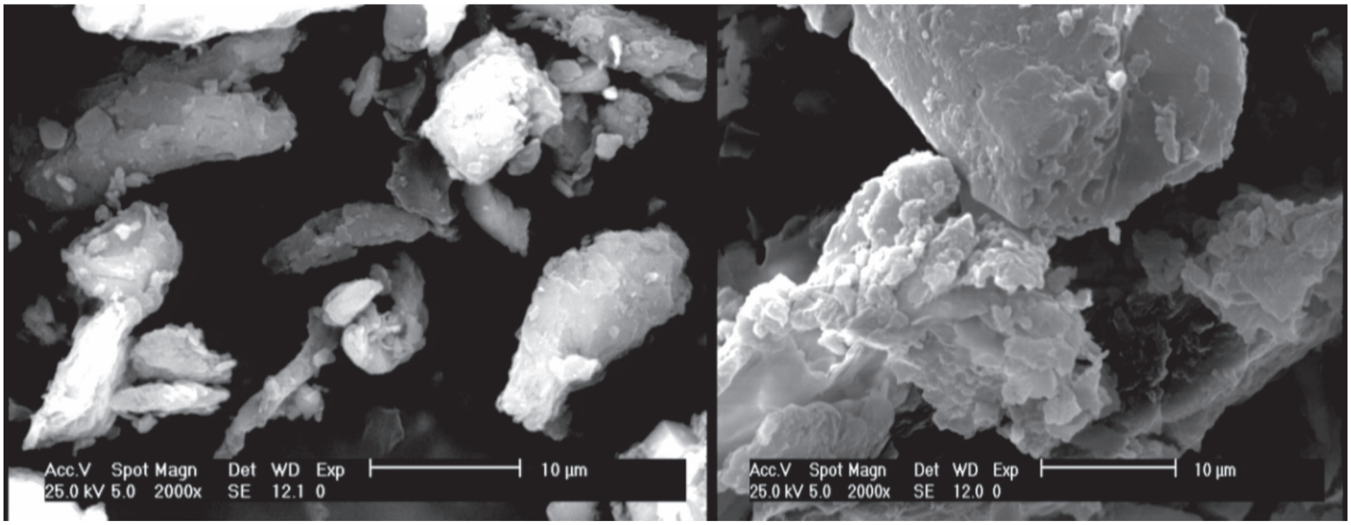


Figure 2. SEM images of used WW sample (a) before and (b) after adsorption process.

Table 1. Textural Properties Obtained by N_2 Adsorption/Desorption.

Parameters	**WW of MWPI**	
BET surface area (m^2/g)	BET[a]	10.70
Pore volume (cm^3/g)	BJH adsorption[b]	0.04705
Pore diameter (Å)	BJH adsorption	27.87

[a]Computed in the P/P0 range 0.05–0.30.

[b]BJH (Barrett Joyner Halenda) cumulative adsorption pore volume for pores in range of 0 and 5000Å diameter.

Adsorption Batch Experiments

A six beaker jar test apparatus was used to simulate the adsorption process. The dye solution and adsorbent were agitated at 150 rpm agitation speed using a jar test. All samples were centrifuged at 5,000 rpm for 10 minutes prior to analysis. Dye concentration of the synthetic wastewater and the real textile wastewater was measured using a HACH spectrophotometer DR/4000 (USA) at a wavelength corresponding to the maximum absorbance of 594 nm (λ_{max}) for RB19. The solution pH was adjusted with 1 M HCl and 1 M NaOH solutions. Also, adsorption studies were carried out at 25 ± 1°C.

RESULTS AND DISCUSSION

Effect of Contact Time on Dye Adsorption

Contact time is one of the most important parameters in the adsorption process. The effect of contact time on RB19 dye adsorption efficiency is displayed in Figure 3. Adsorption efficiency rapidly increased with an increase in contact time within the first minutes. Then, the rate of adsorption was found to be relatively slow and then constant. Similar results have been reported regarding the adsorption process of the same dye on dried pulp and paper sludge [15], the adsorption of crystal violet onto packaging and agricultural greenhouses [16] and adsorption of Direct Nblue_106 on activated carbon from orange peel [17]. As seen in Figure 3, the maximum percentage of RB19 dye removal was obtained at 88.97% at 180 minutes. Results obtained in this step led to the selection of 30 minutes of contact time for further adsorption process experiments.

Effect of Initial pH on Dye Adsorption

The pH of a solution plays an important role in the adsorption process. Experiments for this stage were performed under various ranges of initial pH from 2 to 12 while the initial dye concentration, adsorbent dose and contact time were kept constant at 100 mg/L, 8 g/L and 30 minutes, respectively. Results from this step are displayed in Figure 4. Dye removal efficiency occurred under both acidic and alkaline conditions for the adsorbent (WW). At lower pH levels, maximum observed adsorption capacity may be due to the number of positively charged sites increasing favoring the adsorption of anions due to electrostatic attraction [17].

During lower pH levels, the structure of WW is positively charged. On the other hand, reactive blue 19 (as an anionic dye) is negatively charged. Under acidic conditions, maximum observed adsorption capacity may be due to the electrostatic attractions between negatively charged SO_3^- groups in the dye molecule and positively charged adsorbent's surface.

Lower adsorption at alkaline pH levels versus that of acidic pH levels may be due to presence of excess of OH^- ions competing with dye anions for adsorption sites [17]. Similar trends have been presented by other

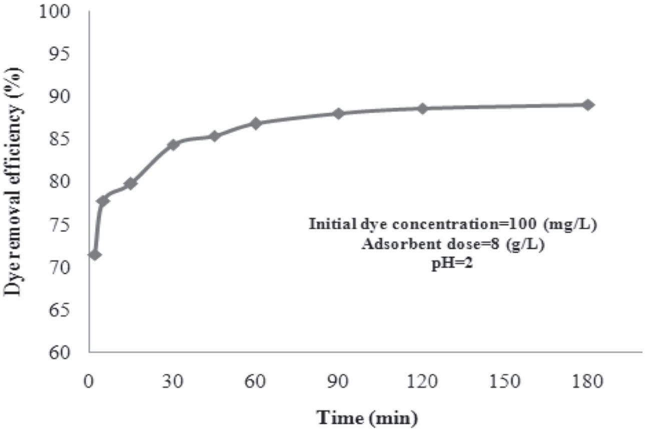


***Figure 3.** Effect of contact time on RB19 adsorption.*

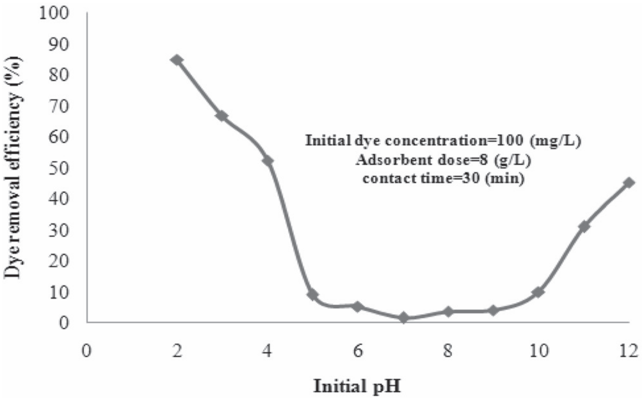


***Figure 4.** Effect of initial pH on RB19 adsorption.*

researchers regarding adsorption of different dye types [15, 17–19]. Displayed in Figure 4, higher dye removal efficiency accrued at a lower pH and the maximum dye removal efficiency was obtained at 84.95% at pH of 2. Dye removal efficiency decreased by increasing the initial pH and reached 1.54% at pH of 7. Then, with an increase of initial pH, the dye removal efficiency increased and reached 45.15% at pH of 12.

In this study, effect from different pH levels (i.e., 2–12) on the stability and dye adsorption of RB19 was investigated. It was quite stable in this range of pH levels observed for RB19 dye. Therefore, the observed dye removal is only the result of the adsorption process.

Effect of Adsorbent Dose on Dye Adsorption

To observe the effect of adsorbent dose regarding RB19 adsorption different amounts of adsorbent dose ranging from 1 to 16 g/L were applied. Experiments were performed under conditions of initial dye concentration at 100 mg/L with a contact time of 30 minutes and at a pH level of 2. The effect of the adsorbent dose on the dye removal efficiency is displayed in Figure 5.

Similar results have been reported for adsorption of Methylene blue onto grass wastes [20], basic blue 9 (BB9) and basic green 4 (BG4) onto modified rice straw [21] and reactive blue 19 onto dried pulp and paper sludge [15]. Increases in percentage of dye removal with adsorbent's dose may be attributed to increases in adsorbent surface areas augmenting the number of adsorption sites available for adsorption [22]. As displayed in Figure 5 maximum and minimum dye removal efficiency was obtained at 22.24% and 93.79% at 1 and 16 g/L doses of WW.

Effect of Initial Dye Concentration on Dye Adsorption

In this step, behavior of RB19 dye adsorption efficiency on WW was evaluated under various initial dye concentrations ranging from 25 to 500 mg/L. Effect of initial dye concentration on RB19 dye removal efficiency is displayed in Figure 6. As displayed in Figure 6, with an increase of initial dye concentration, dye removal efficiency increased at first and then declined. At lower concentrations, the dye concentrations to adsorbent sites ratio is higher which causes an increase in dye removal [15, 23]. Similar trends have been observed by other researchers regarding removal of different types of dyes in different process [15, 23–25]. Dye removal efficiency increased from 83.23% to 85.96% with the increase of initial dye concentration ranging from 25 mg/L to 100 mg/L and then decreased to 49.47 % at the initial dye concentration of 500 mg/L.

Additionally, variations of the amount of dye removed per unit mass of adsorbent (Q) were obtained. It is observed that Q increased with an increase of initial dye concentration and reached from 2.6 to 30.92 mg removed dye/g WW.

Adsorption Isotherms

In order to obtain an interaction between adsorbent and adsorbate, adsorption isotherm studies were conducted with different adsorbent doses ranging from 1 to 16 g/L at a pH of 2 and initial dye concentration of 100 mg/L. In this study, three important isotherms, Langmuir, Freundlich and Temkin isotherms are applied. Isotherms' parameters are presented in Table 2 [26]. Calculated isotherms' constants and correlation

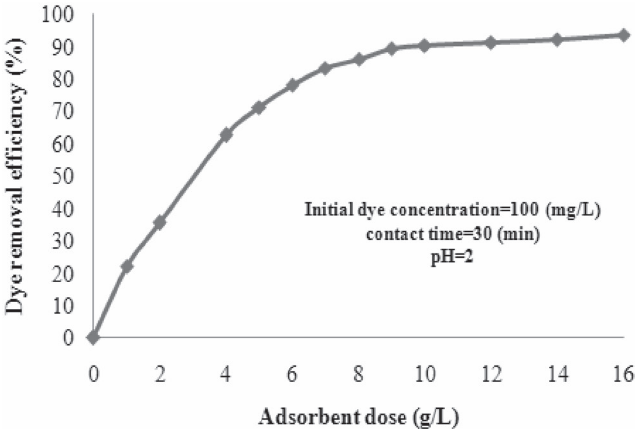


Figure 5. *Effect of adsorbent dose on RB19 adsorption.*

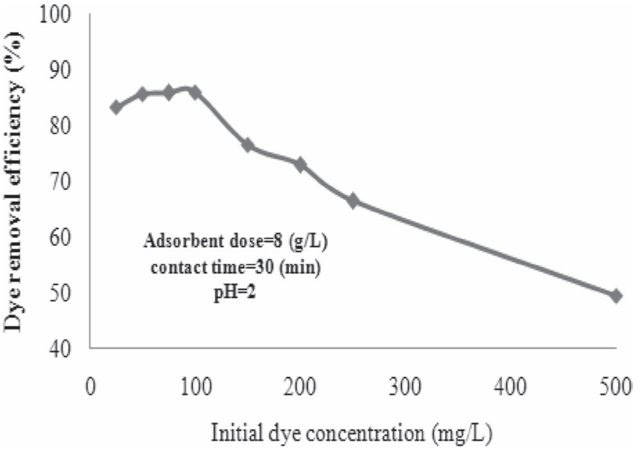


Figure 6. *Effect of initial dye concentration on RB19 adsorption.*

Table 2. Isotherm Equations [26].

Isotherm Name	**Isotherm Equation**		**Parameters**
Langmuir isotherm	$\frac{C_e}{q_e} = \frac{1}{K_L Q_m} + \frac{C_e}{Q_m}$	(1)	C_e = concentration of dye at equilibrium (mg/L) q_e = amount of dye adsorbed per amount of adsorbent at equilibrium(mg/g) K_L = Langmuir adsorption constant (L/mg) Q_m = theoretical maximum adsorption capacity (mg/g)
Freundlich isotherm	$\log q_e = \log K_F + \frac{1}{n} \log C_e$	(2)	K_F = an indicator of the adsorption capacity (L/mg) n = adsorption intensity
Temkin isotherm	$q_e = B_1 \ln K_T + B_1 \ln C_e$	(3)	K_T = equilibrium binding constant (L/mg) B_1 = heat of adsorption

coefficient (R^2) are presented in Table 3. Results suggest the Langmuir adsorption isotherm was the best model for the dye adsorption on WW. In the Langmuir theory, the basic assumption is that sorption takes place at specific homogeneous sites within the adsorbent [27]. In contrast, the adsorption process that followed the Temkin isotherm had an R^2 value of 0.992 in our previous research for adsorption of RB19 dye using dried pulp and paper sludge [15].

Adsorption Kinetics

To investigate the mechanism of sorption, kinetics' parameters for the adsorption process were observed with contact time ranging from 1 to 180 minutes while initial dye concentration, adsorbent dose and pH were kept constant at 100 mg/L, 8 g/L and 2, respectively. Several kinetic models are available for better understanding of the behavior of the adsorbent. Three different kinetic models: the pseudo-first order and pseudo-second order, the Elovich model and intra-particle diffusion model were tested. Kinetics' parameters are provided in Table 4 [26]. The pseudo-first order plot (log ($q_e - q_t$) versus t), the pseudo-second order plot (t/q_t versus t), the Elovich model plot (q versus ln (t)) and the intra-particle diffusion model plot (q_t versus $t^{0.5}$) for the adsorption of RB19 are displayed in Figure 7(a), (b), (c) and (d). Values of kinetics' constants were obtained from the intercept and slope of these plots. Kinetics' constants and correlation coefficient (R2) are presented in Table 5. Results suggest that the adsorption process follows a pseudo-second order model with R^2 value of 0.999.

Table 2. Adsorption Isotherms Constants.

Isotherm Type	**Isotherm Constants**	**R^2**
Langmuir	K_L = 0.04 (L/mg)	0.982
Freundlich	K_F = 2.36 (L/mg), n = 2.14	0.926
Temkin	K_T = 0.38 (L/mg), B_1 = 5.06	0.969

Treatment of Real Textile Wastewater Using WW

Evaluation of real sample textile wastewater containing RB19 dye was also performed under optimum conditions in this study. Optimum values for contact time were 30 minutes, initial pH value of 2 and adsorbent dose at 8 g/L. Initial dye concentration for the real textile wastewater sample was 500 mg/L. Dye removal efficiency of the real wastewater sample was obtained at 73.84%. In addition, Q (i.e., the adsorption capacity) was obtained at 46.1% using WW.

Comparison of Adsorption Capacity of WW with Other Adsorbents

In Table 6, WW from MWPI is compared with other adsorbents based on maximum amount of removed dye per unit mass of adsorbent (i.e., adsorption capacity). Adsorption capacity of the used adsorbent in this study compared with the adsorption capacity of some other adsorbents is acceptable.

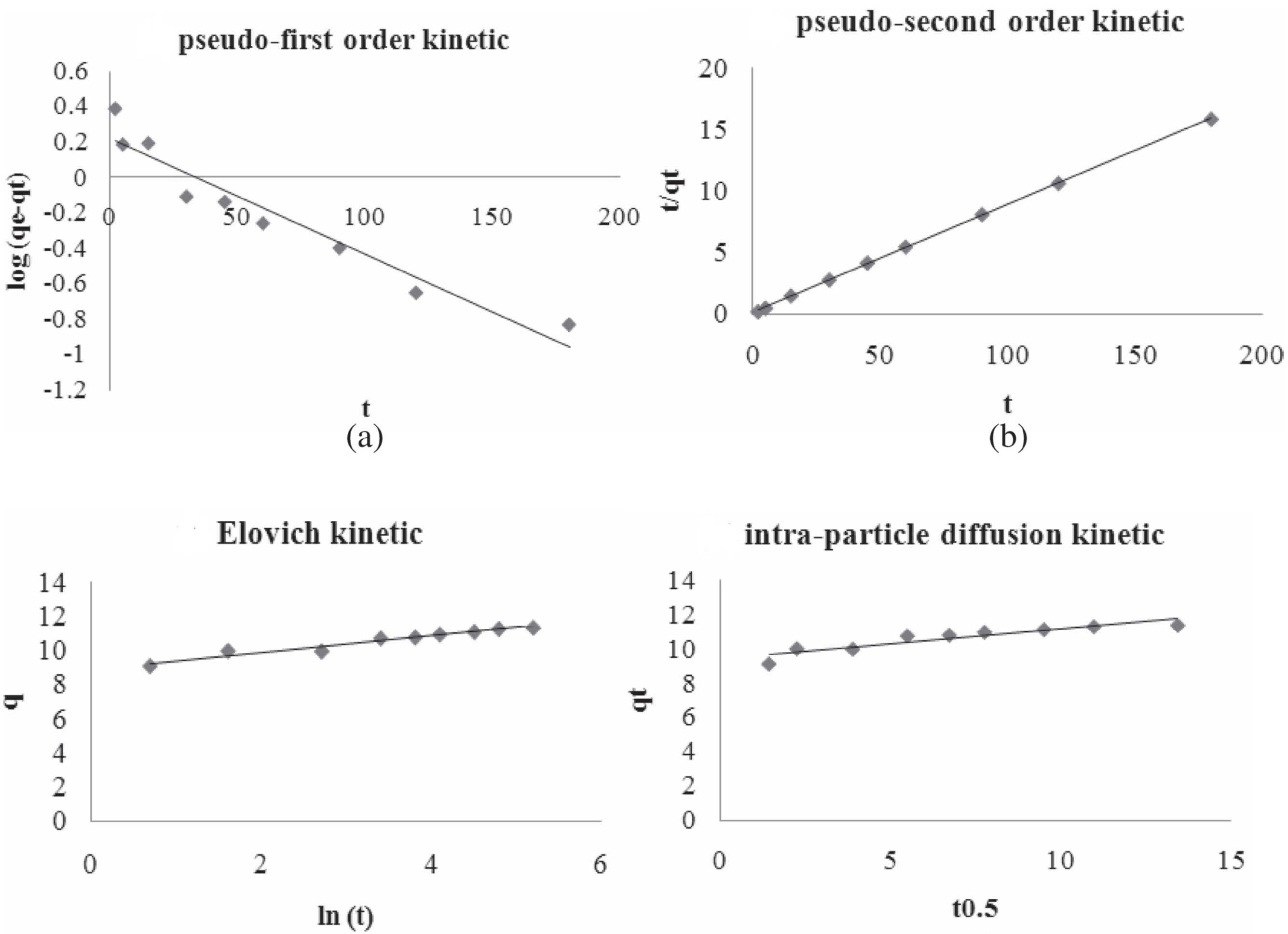
CONCLUSIONS

Performance of WW from MWPI as an efficient, natural and inexpensive adsorbent was investigated for removal of reactive blue 19 dye from aqueous solutions. Summarizing, dye removal efficiency rapidly increased with an increase in contact time during the first minutes and then become constant. Dye removal efficiency occurred in both acidic and alkaline conditions for the adsorbent (WW). Maximum dye removal efficiency was obtained at 84.95% at a pH of 2. When the adsorbent dose was increased dye removal efficiency increased and reached 93.79% at 16 g/L of WW. When the initial dye concentration was increased dye removal efficiency increased at first and then declined. Minimum and maximum removal efficiency was obtained at 49.47% and 85.96% and at an initial dye concentration of 100 mg/L and 500 mg/L,

Table 4. Kinetic Equations [26].

Kinetic Name	Kinetic Equation		Parameters
Pseudo-first order	$\log(q_e - q_t) = \log(q_e) - \dfrac{K_1}{2.303}t$	(4)	q_e = amount of dye adsorbed per amount of adsorbent at equilibrium (mg/g) q_t = amount of dye adsorbed per amount of adsorbent at t (min) (mg/g) K_1 = rate constant (1/min)
Pseudo-second order	$\dfrac{t}{q_t} = \dfrac{1}{K_2 q_e^2} + \dfrac{1}{q_e}t$	(5)	K_2 = rate constant (g/mg.min)
Elovich model	$q_1 = a + b\ln t$	(6)	a, b = constants
Intra-particle diffusion model	$q_t = K_i t^{0.5} + I$	(7)	K_i = intra-particle diffusion rate constant(mg/g.min$^{(1/2)}$) I = the intercept

respectively. Also, the maximum value of Q was obtained at 30.92 mg removed dye/g WW for synthetic wastewater. Among the three isotherms, the Langmuir adsorption isotherm was the best model. Additionally, the adsorption kinetic of RB19 dye was found to follow a pseudo-second-order rate kinetic model with an R^2 value of 0.999. Under optimum conditions for the real textile wastewater containing RB 19 the dye removal efficiency and Q were obtained at 73.84% and 46.1 mg/g, respectively. It is important to note here that adsorption capacities reported in this paper for real and synthetic wastewater provide an acceptable idea of effective adsorbent in comparison to other adsorbents in the adsorption process.



(a) (b)

(c) (d)

Figure 7. *The pseudo-first order plot (a), the pseudo-second order plot (b), the Elovich model plot (c) and the intra-particle diffusion model plot (d) of the adsorption of RB19 for WW.*

Table 5. Adsorption Kinetic Constants.

Isotherm Type	Isotherm Constants	R^2
Pseudo-first order	K_1 = 0.01 (1/min)	0.930
Pseudo-second order	K_2 = 0.05(g/mg.min)	0.999
Elovich model	a = 8.9, b = 0.49	0.951
Intra-particle diffusion model	K_i = 0.172 (mg/g.min$^{0.5}$), I = 9.417	0.836

Therefore, the huge quantity of WW generated by MWPI and its disposal that is causing a significant environmental problem may be considered as alternative, low cost and non-conventional adsorbent for removal of RB19 dye as well as for the real textile wastewater containing RB 19.

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Table 6. Comparison of Adsorption Capacity of Different Adsorbents.

Adsorbents	Dye	Adsorption Capacity (mg/g)	Reference
Lignin	Reactive red HE_3B	10.7	[28]
Modified silk cotton hull waste by H_2SO_4	Reactive blue MR	12.9	[29]
Mansonia wood sawdust	Methyl violet	24.6	[30]
Dried pulp and paper sludge	Reactive blue 19	33.11	[15]
Untreated tendu waste	Congo red	46.95	[8]
Wood apple shell	Crystal violet	95.2	[6]
Orange peel activated Carbon	Direct Nblue_106	107.53	[17]
Modified rice straw	Basic green 4	238.1	[21]
Bambo activated carbon	Reactive black 5	286	[31]
WW of MWPI	Reactive blue 19	30.92	Present study
WW of MWPI	Real textile wastewater (containing RB 19)	46.1	Present study

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Influence of Organic and Inorganic Zinc Sources on Zinc Availability in Soil and its Uptake by Barley

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ABSTRACT: Land application of organic amendments alters soil physico-chemical properties and thereby may affect heavy metal bioavailability. The objective of this study was to investigate zinc (Zn) availability to barley (*Hordeum vulgare* L. cv. Makoei) in a clay loam soil amended with organic and inorganic Zn sources. A field experiment with completely randomized block design was performed with seven treatments in three replicates. A Zn enriched vermi-compost (800 mg Zn kg^{-1}) was applied to the soil at three rates of 25 (V_1), 50 (V_2), and 100 Mg ha^{-1} (V_3). To compare the effects of organic and inorganic sources, similar rates of Zn as $ZnSO_4$ were also applied (Zn_1, Zn_2 and Zn_3, respectively). An untreated soil sample was also considered as control soil (V_0). Results showed that barley plants grown in soil treated with $ZnSO_4$ accumulated significantly greater Zn in their root tissue compared to those grown in soil treated with vermi-compost. A significant increase in the shoot, spike, root, and leaf Zn concentrations was observed as the loading rate of vermi-compost increased. The Zn_1 and Zn_2 treatments caused a significant increase in the Zn spike concentration versus those grown in the control soil. However, at the greatest loading rate of Zn as $ZnSO_4$ (Zn_3), the Zn spike concentration and thereby the 1000-grain weight and the plant height decreased compared to the lower rate of Zn (Zn_2). The results of this experiment point to the fact that the source and the loading rate of Zn are important factors in determining Zn phytoavailability.

INTRODUCTION

THE use of organic amendments as fertilizers and/or soil amendments is becoming increasingly widespread [31]. These amendments contain large amounts of organic matter that can improve the structure of the soil and its water holding capacity. They are also a source of nutrients that improve the fertility and potential productivity of the soil [18]. Nonetheless, concerns regarding their content in potentially hazardous heavy metals such as zinc still persist. If excessive loads of pollutants are introduced with the application of low-quality waste, soil fertility may be adversely affected, ground-water quality threatened, and the food chain contaminated [14, 20]. The introduction of heavy metals to soil and their transfer to crops, therefore, needs to be controlled. However, in arid ecosystems, the probability of nutrients or toxic elements leaching into ground-water is low [29]. On the other hand, sustainable agriculture has become a major issue in the world. More and more farmers in different countries are now cultivating their land without using chemical products, pesticides, and herbicides [10]. However, deficiency of nutrients such as N and P, are the principal constraints to crop production under low input agricultural systems. Agronomic practices aimed at reducing the dependence on chemical fertilizers need to incorporate crop residues or other forms of organic material, thus providing nutrients and thereby improving soil structure and fertility [26].

Metal bioavailability in soil from organic or inorganic amendments varies widely and is affected by many soil parameters such as pH and type of soil minerals [5].

Evaluation of phytoavailability of organic amendment metals has illustrated that metals added to organic amendment are less phytoavailable than metal salts added to the soils without organic amendment [7, 12, 15]. The amount of metals required to cause phytotoxicity or yield reductions are always less with salts as compared with organic amendment. The phytoavail-

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ability of metals is normally higher in the first year of organic amendment application, then it decreases with time after application has ceased, and stays at a lower level for an extended time [15]. These results indicate that organic amendments add adsorptive phases to soil, which may affect metal bioavailability to plants [15].

Organic amendments such as sewage sludge or cow manure often contain pathogens that restrict their application to agricultural soil [10]. There are different techniques to reduce pathogens, such as liming to pH 12, but there is a growing tendency to the use of compost or vermi-compost organic wastes [4]. Vermi-composting is the non-thermophilic biodegradation of organic material through interaction between earthworms and microorganisms [17], where organic material residuals are fragmented rapidly into much finer particles by passing through a grinding gizzard, while maintaining nutrients [23]. Earthworms reduce the number of human pathogens, an effect obtained in traditional composting by increasing temperature [10].

Addition of organic amendments to soil not only provides nutrients to crops but also improves soil physico-chemical properties and may affect metal bioavailability. Hence, there is an increasing interest in utilization of organic amendments (such as vermi-compost) as a fertilizer. This research was conducted to evaluate Zn availability to barley (cv. Makoei) in a clay loam soil amended with organic (Zn enriched vermi-compost) and inorganic ($ZnSO_4$) Zn sources. The influence of various Zn sources and loading rates on the plant height and 1000-grain weight in barley cultivated in organic and inorganic amended soils was also investigated.

MATERIALS AND METHODS

Experimental Design

The field experiment was conducted at Arak University research farm, central Iran (34º 05′ 21″ N, 49º 42′ 36″ E), during 2009–2010 cropping seasons. The experimental soil of the plot was a fine, mixed, mesic, Typic Haploxerepts [33] and had high pH, low organic carbon content, and low available Zn concentration (Table 1).

The mean annual temperature and rainfall were 13.8°C and 324.3 mm, respectively. Four soil cores (0–20 cm depth) were taken from each plot and were mixed to make a composite sample. Soil samples were air-dried, sieved (2 mm), and prepared for chemical analysis. Some of the physico-chemical properties of the soil are presented in Table 1.

Table 1. Selected Physico-chemical Properties of Unamended Soil.

Parameter	Unit	Unamended soil
pH	–	7.9
EC	dS m^{-1}	1.3
Sand	%	30
Silt	%	35
Clay	%	35
Organic carbon	%	0.6
Total P	%	0.01
$CaCO_3$	%	10
DTPA-extractable Zn	mg kg^{-1}	ND*
CEC	cmolc kg^{-1}	11.3

*ND = non-detected

Vermi-compost with a low level of heavy metal (i.e. Pb, Cd) concentration was selected from Qom province, center of Iran. As in previous researches [8, 21], the vermi-compost was enriched with $ZnSO_4$ to 800 mg Zn kg^{-1} and incubated for two weeks. Selected properties of the organic amendment are shown in Table 2. The concentration of the metals in the vermi-compost was less than their tolerable standard values [37].

Three rates of Zn (12, 24, and 48 g Zn $plot^{-1}$) in the form of vermi-compost and $ZnSO_4$ were used in the soil. Accordingly, three loading rates of Zn enriched vermi-compost including 25, 50, and 100 Mg ha^{-1} (V_1, V_2 and V_3, respectively) and three rates of $ZnSO_4$ including 49.5, 99, and 198.1 kg ha^{-1} (Zn_1, Zn_2 and Zn_3, respectively) were used. In addition, an untreated soil was considered as the control soil (V_0). All of the treatments were replicated three times. After adding the vermi-compost, the soil moisture was kept at 80% water holding capacity during the incubation for two weeks.

Seeds of barley (*Hordeum vulgare* L. cv. Makoei) were drill-seeded on October 19, 2009 by hand at a seeding rate of 450 plants m^{-2}. The plots were irrigated

Table 2. Selected Properties of the Applied Vermi-compost.

Characteristic	Unit	Vermi-compost	U.S. EPA Regulations
pH	–	7.3	–
EC	dS m^{-1}	17.2	–
Organic carbon	%	13.2	–
N	%	1.5	–
Pb	mg kg^{-1}	2.5	–
Zn*	mg kg^{-1}	241.7	2800
Ni	mg kg^{-1}	2.9	420
Cd	mg kg^{-1}	0.4	39
Cr	mg kg^{-1}	19.5	3000

*before enrichment

using well water (with pH range of 7.4–7.8 and EC nearly 1 dS m^{-1}).

On May 29, 2010, after harvesting the plants, the roots, shoots, spikes, and leaves were sampled separately. The plants were oven-dried at 65ºC to a constant weight, for 24 h and the dry matter weight was recorded. Thereafter, plant samples were combusted overnight at 480ºC

The 1000-grain weight was calculated by measuring the weight of 100 seeds and multiplying it by ten.

Analysis

Plant Analysis

After the plant samples were combusted overnight at 480°C, the ash was treated with HNO_3 and heated to near dryness, and the samples dissolved in 3 M HC1 with heating. Zn concentration in the extracted solutions was measured using atomic absorption spectrophotometry (AAS) (Perkin Elmer model 3030).

Soil Analysis

Total Zn in soil and vermi-compost was extracted using tri-acid mixture (HNO_3:H_2SO_4:$HClO_4$ 5:1:1) and then determined on atomic absorption spectrophotometry (AAS) [1]. The DTPA-extractable Zn was extracted using 0.005 M DTPA, 0.1 TEA, and 0.01 M $CaCl_2$, adjusted to pH 7.3 [16]. Soil pH was measured with a digital pH meter (Model 691, Metrohm AG Herisau Switzerland) [36] and electrical conductivity (EC) was measured with an EC meter (Model Ohm-644, Metrohm AG Herisau Switzerland) [28]. Organic matter content was determined by the Walkley and Black method [24]. Soil texture was determined according to a method presented by Gee and Bauder (1986) [9]. Total soil P was determined by a colorimetric method [25]. Cation exchange capacity (CEC) was measured using the Rhoades (1982) method [27].

Translocation Factor (TF)

The ability of plants to transport heavy metals from roots to shoots was measured by calculating translocation factor (TF) as displayed in Equation (1) [31]:

$$TF = H_s / H_r \quad (1)$$

where H_s and H_r are heavy metal concentrations in shoot and root, respectively.

Quality control

The accuracy of Zn analysis was controlled by analyzing certified standards of National Institute of Standards and Technology (NIST) and including blanks in digestion batches. The results were found to be within ±2% of the certified value. Quality control measures were taken to assess the contamination and reliability of data. The coefficient of variation of replicate analysis was determined for precision. Variations were found to be less than 10%.

Statistics

A completely block randomized design in three replications was used. The statistical analyses of data were performed using the ANOVA procedure [30]. Differences between means were evaluated using the least significant difference (LSD). The 0.01 probability value was used to determine significant difference.

RESULTS AND DISCUSSION

Organic Amendment Effects on Soil Properties

Zn sources and different loading rates of Zn had no significant effect on soil pH (Table 3). Atiyeh et al. (2001) reported that the addition of pig manure vermi-compost with pH = 5.7 decreased pH progressively as the application rate of vermi-compost increased [2]. The stability in pH in our mixtures can be due to the buffer capacity of the soil used [11].

The electrical conductivity (EC) of the Zn enriched vermi-compost was 17.2 dS m^{-1} (Table 2) which is lower than reported elsewhere in the literature [2, 19]. Masciandaro et al. (2000) [19] reported an EC of 46.7 dS m^{-1} for vermi-compost derived from sewage sludge. Atiyeh et al. (2001) [2] reported 32.2 dS m^{-1} for pig manure vermi-compost. The EC of the vermi-compost depends on the raw materials used for vermi-composting and is related to their ion concentration [3]. The soil EC increased with increasing the loading rate of Zn enriched vermi-compost (Table 3). Increased soil EC in organic amended soils is due to the high level of soluble salts in the vermi-compost. Increased soil EC may result in some detrimental effects on plants and microorganisms.

Soil application of Zn enriched vermi-compost (V_1, V_2 and V_3) increased the soil cation exchange capacity (CEC) by 0.9, 1.4, and 2.2 units with increasing soil organic carbon by 0.2%, 0.4%, and 0.8% compared to

Table 3. Influence of Various Zn Sources and Their Loading Rates on Soil Properties.

Treatment	Organic carbon (%)	CEC (cmolc kg^{-1})	EC (dS m^{-1})	pH
Zn_1	0.6^{d*}	11.3^{d}	1.4^{e}	7.8^{a}
Zn_2	0.6^{d}	11.3^{d}	1.5^{e}	7.8^{a}
Zn_3	0.6^{d}	11.3^{d}	2.5^{d}	7.8^{a}
V_0	0.6^{d}	11.3^{d}	1.3^{e}	7.9^{a}
V_1	0.8^{c}	12.2^{c}	2.9^{c}	7.8^{a}
V_2	1.0^{b}	12.7^{b}	3.4^{b}	7.9^{a}
V_3	1.4^{a}	13.5^{a}	4.1^{a}	7.8^{a}

*Means followed by the same letter in each column are not significantly different (P = 0.01).

V_1, V_2, and V_3 were Zn enriched vermi-compost at doses of 25, 50, and 100 Mg ha^{-1}. Zn_1, Zn_2, and Zn_3 were the similar Zn content of Zn enriched vermi-compost (V_1, V_2 and V_3, respectively) supplied from $ZnSO_4$. V_0 was considered as control.

the control soil (V_0), respectively (Table 3). Increasing the CEC of soil amended with vermi-compost increases the adsorption capacity of the soil and thus changes the solubility of the metal [5].

Zn Concentration in Plant Tissues

Application of Zn as both vermi-compost and $ZnSO_4$ significantly increased the tissue Zn concentration of barley plants compared to the plants cultivated in the control soil (V_0) (Table 4). The root Zn concentration in plants grown in the plots treated with vermi-compost was lower than that of plants grown in the plots which received $ZnSO_4$. Increasing the loading rate of Zn as $ZnSO_4$ (Zn_1, Zn_2 and Zn_3) significantly increased the root Zn concentrations by 2.1, 2.5, and 5.2 times, while the application of Zn enriched vermi-compost (V_1, V_2 and V_3), significantly increased them by 1.6, 2.2, and 1.6 times compared to those grown in the control soil, respectively (Table 4). Similar to this result, other researchers have reported that the metals applied by inorganic salt are more phytoavailable than the equivalent quantity of metal applied by organic amendments [5, 7, 12]. These results have been interpreted as indicating that organic amendment adds adsorptive phases to soils, which reduce metal availability to plants [5].

Table 4. Influence of Various Zn Sources and Their Loading Rates on Zn Concentration in Barley Tissues (mg kg^{-1}).

Treatment	Shoot	Root	Spike	Leaf
V_0	ND**	18.4^{f*}	ND	ND
Zn_1	7.1^{e}	38.6^{d}	8.2^{f}	14.1^{d}
Zn_2	24.1^{b}	45.8^{b}	20.7^{b}	16.0^{c}
Zn_3	27.8^{a}	96.4^{a}	18.0^{c}	25.1^{a}
V_1	7.1^{e}	29.4^{e}	11.2^{e}	14.6^{d}
V_2	10.8^{d}	40.1^{c}	15.1^{d}	16.3^{c}
V_3	15.0^{c}	30.2^{e}	22.4^{a}	18.0^{b}

*Means followed by the same letter in each column are not significantly different (p = 0.01).

**ND = non-detected. V_1, V_2, and V_3 were Zn enriched vermi-compost at doses of 25, 50, and 100 Mg ha^{-1}. Zn_1, Zn_2, and Zn_3 were the similar Zn content of Zn enriched vermi-compost (V_1, V_2 and V_3, respectively) supplied from $ZnSO_4$. V_0 was considered as control.

The root Zn concentrations in barley plants were much higher than those in aerial tissues. Partitioning and distribution of trace elements in different plant tissues depends on the plant species [38].

Increasing the loading rate of $ZnSO_4$ from 49.5 (Zn_1) to 99 and 198.1 kg ha^{-1} (Zn_2 and Zn_3) significantly increased the shoot Zn concentrations by 3.4 and 3.9 times, while increasing the loading rate of Zn enriched vermi-compost from 25 to 50 and 100 Mg ha-1, significantly increased the shoot Zn concentrations by 1.5 and 2.1 times, respectively. Similar to the root Zn concentration, the shoot Zn concentration of the plants grown in organic amended soil (V_2 and V_3) was lower than that of plants grown in inorganic amended soil (Zn_2 and Zn_3), respectively (Table 4) .

Increasing the applied rate of $ZnSO_4$ from 49.5 (Zn_1) to 99 (Zn_2) kg ha^{-1} significantly increased the spike Zn concentration by 2.5 times (Table 4), while decreased it at the greatest loading rate of Zn (Zn_3). The significant decrease of the 1000-grain weight at the Zn_3 treatment (Table 5) may be due to the significant decrease of the spike Zn concentration at the Zn_3 treatment compared to Zn_2 treatment. On the other hand, 2.5 times increasing in the spike Zn concentration of Zn_2 compared to Zn_1 treatment resulted in a significant increase in the 1000-grain weight of barley by 4.7 g (Table 5).

Table 5. Influence of Various Zn Sources and Their Loading Rates on Plant Height and the 1000-grain Weight in Barley Cultivated in Organic and Inorganic Amended Soils.

Treatment	Plant Height (cm)	1000-grain weight (g)
V_0	66.1^{f*}	33.5^{e}
Zn_1	72.0^{e}	36.7^{d}
Zn_2	84.7^{b}	41.4^{b}
Zn_3	73.5^{d}	37.3^{d}
V_1	81.3^{c}	38.8^{c}
V_2	86.0^{b}	44.6^{a}
V_3	94.3^{a}	44.0^{a}

*Means followed by the same letter in each column are not significantly different (p = 0.01).

V_1, V_2, and V_3 were Zn enriched vermi-compost at doses of 25, 50, and 100 Mg ha^{-1}. Zn_1, Zn_2, and Zn_3 were the similar Zn content of Zn enriched vermi-compost (V_1, V_2 and V_3, respectively) supplied from $ZnSO_4$. V_0 was considered as control.

Applying Zn enriched vermi-compost significantly increased the spike Zn concentration with the greatest increase at the V_3 treatment (Table 4). The Zn spike concentration ratio of V_3 to V_2 treatment was significantly greater than that of V_2 to V_1 treatment. It may be concluded that applying the greatest loading rate of vermi-compost in the soil increased the solubility of Zn, thereby increased spike Zn concentration. Accordingly, the Zn concentration ratio of spike to shoot in the plants grown in vermi-compost treated soils was more than 1 (Table 6). Bourg (1992) and Stevenson (1982) reported that organic metal complexes in soil solution play an important role in the soil Zn availability to plants [6, 35]. The greatest Zn spike concentration was found at the V_3 treatment (Table 4).

Except for the greatest loading rate of Zn (V_3 and Zn_3), at similar loading rates, leaf Zn concentration did not show any significant difference in the plants grown in the organic amended soil and those grown in the $ZnSO_4$-treated soil (Table 4). The leaf Zn concentration in the plants grown in the soil treated with 100 Mg ha^{-1} vermi-compost was significantly lower than in those that received the same level of Zn from $ZnSO_4$.

Increasing the loading rate of $ZnSO_4$ from 49.5 (Zn_1) to 99 kg ha^{-1} (Zn_2) significantly increased the Zn concentration ratios of spike to leaf and spike to root by nearly 2 times and decreased the Zn concentration ratio of spike to shoot by 1.3 times (Table 6).

Applying the greatest loading rate of Zn as $ZnSO_4$ (Zn_3) caused a significant decrease in the Zn concentration ratios of spike to shoot, spike to leaf, and spike to root compared to the lower rate of Zn (Zn_2) treatment. Accordingly, the 1000-grain weight of barley plants at the Zn_3 treatment was significantly lower than the Zn_2 treatment.

Table 6. Influence of Various Zn Sources and Their Loading Rates on Zn Concentration Ratios of Spike to Stem, Spike to Leaf, and Spike to Root.

	Zn concentration		
Treatment	**Spike to leaf**	**Spike to shoot**	**Spike to root**
Zn_1	0.58^{d*}	1.16^{c}	0.21^{d}
Zn_2	1.29^{a}	0.86^{d}	0.45^{b}
Zn_3	0.72^{c}	0.65^{e}	0.19^{e}
V_1	0.77^{c}	1.57^{a}	0.38^{c}
V_2	0.93^{b}	1.40^{b}	0.38^{c}
V_3	1.24^{a}	1.50^{a}	0.74^{a}

*Means followed by the same letter in each column are not significantly different (p = 0.01).

V_1, V_2, and V_3 were Zn enriched vermi-compost at doses of 25, 50, and 100 Mg ha^{-1}. Zn_1, Zn_2, and Zn_3 were the similar Zn content of Zn enriched vermi-compost (V_1, V_2 and V_3, respectively) supplied from $ZnSO_4$.

Increasing the loading rate of the Zn in the form of vermi-compost caused a significant increase in the Zn concentration ratio of spike to leaf. Accordingly, the spike Zn concentration at the V_3 was greater compared to the Zn_3 treatment (Table 4).

Translocation Factor (TF) of Zn

The ability of plants to translocate heavy metals from roots to shoots is measured by calculating the translocation factor. TF values of more than 1, suggest that heavy metals are readily transported from root to shoot whereas values less than 1 signify more accumulation of heavy metals in root.

Zn concentration in the aerial parts of the plants cultivated in the control soil (V_0) was less than the detection limit of the atomic absorption spectrophotometry (Table 4).

Regardless of the Zn source, the TF value of Zn was less than 1 for all treatments (Table 7). Singh and Sinha (2005) reported increased accumulation of Zn in tissues of *B. Juncea* with increasing rate of tannery sludge from 10 to 100%, although the greatest accumulation of Zn was found in roots [32].

The TF ranged from 0.18 to 0.53 with the greatest values of 0.53 and 0.49 for inorganic (Zn_2) and organic (V_3) treatments, respectively (Table 7). At the greatest loading rate of Zn as $ZnSO_4$, the TF value significantly decreased. Accordingly, the plant height and 1000-grain weight of barley plants at the Zn_3 treatment were significantly lower than those at the Zn_2 treatment (Table 5). Increasing the loading rate of vermi-compost significantly affected the translocation factor. It can be concluded that applying enriched vermi-compost increased the plant growth and thereby increased the TF value.

Table 7. Influence of Various Zn Sources and Their Loading Rates on Zn Translocation Factor (TF).

Treatment	TF
Zn_1	0.18^{f*}
Zn_2	0.53^{a}
Zn_3	0.29^{c}
V_1	0.24^{e}
V_2	0.27^{d}
V_3	0.49^{b}

*Means followed by the same letter in each column are not significantly different (p = 0.01).

V_1, V_2, and V_3 were Zn enriched vermi-compost at doses of 25, 50, and 100 Mg ha^{-1}. Zn_1, Zn_2, and Zn_3 were the similar Zn content of Zn enriched vermi-compost (V_1, V_2 and V_3, respectively) supplied from $ZnSO_4$.

Effect of Organic and Inorganic Zn Sources on Growth and Yield Attributes

Application of Zn from both organic and inorganic sources significantly increased the plant height and the 1000-grain weight of barley plants (Table 5). Applying the greatest loading rate of Zn as $ZnSO_4$ (Zn_3) significantly decreased the plant height and the 1000-grain weight compared to the lower rate of applied Zn (Zn_2); that may be due to the Zn toxicity effect on plant growth [13]. However the assessment of toxic concentrations and the effects of trace elements on plants is very complex, because it depends on so many factors that cannot be measured on a linear scale [13].

Applying Zn enriched vermi-compost to the soil at the rates of 25 and 100 Mg ha^{-1} caused a significant increase in the plant height by 13% and 28.2% compared to the similar loading rate of Zn inorganic as $ZnSO_4$, respectively (Table 5). Increasing the loading rate of Zn in the form of vermi-compost (V_1, V_2 and V_3) caused a significant increase in the 1000-grain weight compared to Zn inorganic treatments (Zn_1, Zn_2 and Zn_3) by 2.1, 3.2, and 6.7 g, respectively. It can be concluded that plant growth is positively affected by the soil organic carbon content. Speir et.al. (2004) reported that increasing organic amendments caused a significant increase on soil biological characteristics such as soil respiration, biomass and increasing the enzyme activity [34]. In addition to improving soil quality, land application of organic amendments enables recycling of valuable components such as organic matter (OM) and plant nutrients. Improvements in nutritional status of soil due to organic amendments have been reported widely [22, 31].

Effect of Organic and Inorganic Zn Sources on the Soil DTPA-extractable Zn

Increasing the loading rate of Zn from both organic and inorganic sources significantly increased the soil DTPA-extractable Zn. At the same loading rate, the DTPA-extractable Zn in the soils treated with $ZnSO_4$ was significantly greater than the vermi-compost amended soils (Figure 1). Applying organic Zn (V1, V_2 and V_3) significantly decreased the Soil DTPA-extractable Zn by 13%, 7.6%, and 33.4% compared to the similar rate of Zn inorganic as $ZnSO_4$ (Zn_1, Zn_2 and Zn 3), respectively. Similar to our results, Li et al. (2001) and Hettiarachchi et al. (2003) showed that the amount of metals required to cause yield reductions are always less with salts as compared to organic amendments [12, 15]. The organic residuals generally contain different compounds such as organic materials, Fe, Mn, and Al oxides which adsorb heavy metals and could reduce the solubility of metals in the polluted soils [5].

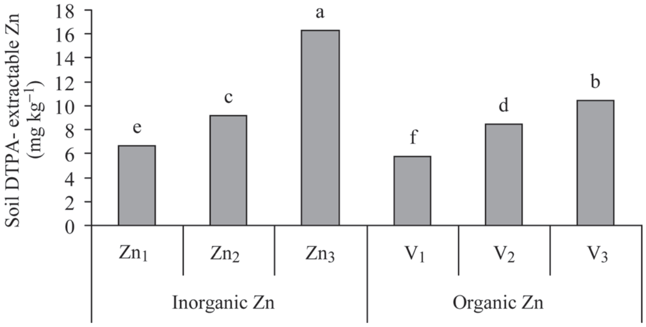

Figure 1. *Influence of organic and inorganic Zn sources and their loading rates on soil DTPA- extractable Zn concentration. Means followed by the same letter are not significantly different (p = 0.01). V_1, V_2, and V_3 were Zn enriched vermi-compost at doses of 25, 50, and 100 Mg ha^{-1}. Zn_1, Zn_2, and Zn_3 were the similar Zn content of Zn enriched vermi-compost (V_1, V_2 and V_3, respectively) supplied from $ZnSO_4$.*

CONCLUSION

Results revealed that Zn-phytoavailability in soils treated with vermi-compost was significantly lower than those treated with $ZnSO_4$ salt. Greater decreases of soil DTPA-extractable Zn at the V_3 treatment compared to the Zn_3 treatment leads one to conclude that land application of vermi-compost increased adsorption capacity of the soil and thus decreased soil DTPA-extractable Zn. Application of Zn from both organic and inorganic sources significantly increased plant height and the 1000-grain weight of barley plants. However, the plant height and the 1000-grain weight of barley significantly decreased during the greatest loading rate of Zn as $ZnSO_4$. Increasing the greatest loading rate of Zn from vermi-compost significantly increased plant height and the 1000-grain weight of barley compared to lower rates of Zn. This suggested that plant growth in organically amended soil maybe affected by soil organic carbon content and plant nutrients available in vermi-compost. Therefore, it appears applying the greatest loading rate of Zn enriched vermi-compost may have significant effects on plant growth and Zn spike concentration.

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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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