ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

MICROBIOLOGY

EFFECT OF DIFFRENT AMOUNT OF SALTS ON DETOXIFICATION AND SOME CHEMICAL PROPERTIES OF MUNICIPAL SOLID WASTE LEACHATE

[1*]MEHRNOUSH ESKANDARI TORBAGHAN, ALIREZA ASTARAEI AND [2]MASOUD ESKANDARI TORBAGHAN

[1.] Soil and Water expert of North Khorasan Agricultural and natural Resource Research Center & member of Young Researchers Club (Islamic Azad University of Bojnord), Bojnourd-IRAN
2. Associate Professor of Soil Science Department, Faculty of Agriculture, Ferdowsi University
[3.] Academic member of North Khorasan Agricultural and Natural Resource Research Center, Shirvan-IRAN

MEHRNOUSH ESKANDARI TORBAGHAN
Soil and Water expert of North Khorasan Agricultural and natural Resource Research Center & member of Young Researchers Club (Islamic Azad University of Bojnord), Bojnourd-IRAN

*Corresponding author

ABSTRACT

Water is the main part of municipal sewage comprising 99 to 99.5 percent of sewage. Since, MS water and MSW leachate are having high EC and toxicity effects; water treatment with chemicals is used to decrease microbial contamination and toxicity. Coagulants such as ferric chloride, alum, etc. are usually used in coagulation as a common process in water treatments. A laboratory experiment was performed to study the effects of three different coagulants of copper sulfate, ferric chloride and sodium benzoate in two levels of 40 and 80 mg l^{-1} on microbial detoxification and on chemical properties of municipal solid waste leachate. Electrical conductivity and total soluble organic carbon decreased in all treatments if compared to control (pure leachate).Solutions of 40 mg l^{-1} ferric chloride, 40 and 80 mg l^{-1} sodium benzoate were more efficient in reducing total number of microorganisms. Copper sulfate at 40 mg l^{-1} had greater effect in reducing number of fecal and non fecal coli forms whereas, sodium benzoate had maximum reduction in number of fungi at 40 mg l^{-1}.

ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

KEY WORDS

Chemical salts, Detoxification, Salinity, pH, Organic carbon, Municipal solid waste leachate

INTRODUCTION

Municipal waste has increased as result of increasing population and migration of people to cities. Recycling of municipal waste into compost is considered as a management method more advantageous than burning or disposing them off in some sites. Unlike other countries, urban waste produced in most of the cites in Iran contains about 70% moisture. Leachate produced during recycling of solid waste into compost is very high and usually left in natural reservoirs resulting in soil, water and environmental pollutions .Water treatment is used to decrease microbial and chemical contaminations of water. Coagulation, deposition, filtering and detoxification are the common process in water and waste water treatments. Deposition of coagulants via coagulation method is the only method, common in water and waste water treatment. Coagulants are metal salts, such as, aluminum sulfate (Alum), ferric sulfate, ferro sulfate, and ferric chloride, organic anion, organic cation and organic non ionic polymers [1]. Researcher [2] concluded that ferric chloride compared of alum was more effective in removal color, turbidity and COD. Many research works on coagulants such as alum and ferric chloride conducted in Canada, USA, China, Italy and France remove water turbidity with chemical, biological and physical means[3]. Municipal liquid and solid wastes leachates are very rich sources of nutrients and can be used as a source of liquid fertilizer to improve soil fertility and its physicochemical properties, thereby, maintaining soil quality. Safe use of these resources requires special management practices followed by standard and accepted national and international methods to safeguard water, soil and environment. Steven et al. [4] reported that those substrates, whose digestibilities were most affected, were those that are insoluble in water and are known to form complexes with iron or aluminum. Thus, amino acids, proteins and long-chain fatty acids were particularly affected, while glucose and butyric acid were not and cellulose was moderately affected. Marthakutty et al.[5], on antimicrobial studies of copper complexes with Cl^-, Br^-, NO_3^-, NCS^-, N_3^- and SO_4^{-2} ligands, realized that the complexes were found to be active against *Bacillus sp*. *Vibrio cholera OI*, *Staphylococcus aurus* and *Salmonella paratyghi* . Report of Ogiehor et al. [6] on antimicrobial effects of sodium benzoate concentrations on the growth, survival and aflatoxin production of *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*, showed that when sodium benzoate concentration increased from 0.2 to 0.6 percent, the number of live fungi decreased in all three species. Lopez-Malo et al. [7] concluded that spore germination time and radial growth rates (RGR) of *Aspergillus flavus in* P.D.A. culture medium significantly decreased with the presence of antimicrobial agents such as sodium benzoate. *Aspergillus flavus* germination time increased and its growth decreased as antimicrobial agents concentration increased. This work was undertaken to examine the effect of different amount of salts (copper sulfate, ferric chloride and sodium benzoate) on detoxification and some chemical properties of municipal solid waste leachate.

MATERIALS AND METHODS

A laboratory experiment was conducted to study the effect of copper sulfate, ferric chloride and sodium benzoate on some chemical

properties and microbial detoxification of municipal solid waste leachate (MSWL) in Mashhad city. Four salt treatments were selected: 1) control (untreated MSWL (Table 1), L); 2) MSW leachate + copper sulfate (Lcs); 3) MSW leachate +ferric chloride (Lfc); 4) MSW leachate + sodium benzoate (Lsb). Each salt was present with two levels of 40 and 80 mg l^{-1} as sub-treatments [1, 8, 6] in a Completely Randomized Design (factorial). Three replications were performed.

Table1
Chemical properties of municipal solid waste leachate

Variable	Unit	Amount
pH	-	5.97
EC	(dSm^{-1})	29.43
TOC	(mg Kg^{-1})	12948
Dry Matter	(%)	2.5
Total Nitrogen	(%)	0.175
Phosphorus(available)	(%)	12.94
K	(%)	0.27
CL	(%)	0.45
$SO_4^=$	(%)	0.33
Fe	(mg Kg^{-1})	155.48
Mn	(mg Kg^{-1})	15.1
Zn	(mg Kg^{-1})	30.43
Cu	(mg Kg^{-1})	1.89

Salts in two concentrations (40 and 80 mg l^{-1}) were added to each plastic containers having one liter of pure MSW leachate with three replications. All plastic containers were placed on a mechanical shaker for one hour at 60 rpm speed. Thereafter, they are kept for 48 hours at room temperature for sedimentation. Sampling from each container was done from supernatant solution and electrical conductivity, pH and soluble organic carbon were determined by EC meter, pH meter and titration with ammonium ferro sulfate [9] respectively. To determine the bacterial number [10] , fecal and non fecal coli forms numbers [11] and fungi number [10],three culture mediums was prepared: (Plate Count Agar) (P.C.A.), (Eosine Demethylene Blue Agar)(E.M.B.), (Domme Terre Agar) (P.D.A.) ,respectively. Sampling from cultures was performed in two methods of Surface Inoculant and Pour Plate [11]. Inoculated Petri dishes were kept in incubator for 48 hours at 25 °C. Identification, isolation and counting of microorganisms were performed with colony counter [12] and the total number of microorganisms was calculated. Data was analyzed statistically by MSTAT-C and the data means were compared by Duncan's Multiple Range Test at P=5%.

RESULTS

Table 2 showed that 40 and 80 mg l^{-1} copper sulfate treatments and 80 mg l^{-1} sodium benzoate treatment have a significant change in the pH of leachate compared to other treatments .Acidity in these treatments decreased significantly (-11.9,-12.2 and -11.4% respectively) compared to control (p=0.05).
Electrical conductivity was reduced significantly in ferric chloride 40 mg l^{-1} (-60.3%) and 80 mg l^{-1}

(-59.5%) compared to control, as for the electrical conductivity of MSW leachate in 40 and 80 mg l^{-1} copper sulfate and 80 mg l^{-1} sodium benzoate (Table 2). Since electrical conductivity of root zone has a vital role in cultivated plants in soil, reduction in EC of leachate is important in promoting plant growth.

Chemical salts (copper sulfate, ferric chloride and sodium benzoate) decreased the amount of soluble organic carbon in all treatments when compared to control. Maximum soluble organic carbon reduction was recorded in 40 mg l^{-1} ferric chloride followed by 40 mg l^{-1} sodium benzoate treatments, having -22% and -18% reductions respectively when compared to pure MSW leachate (control), (Table 2).

Table 2

Effect of experimental treatments on pH, EC and Soluble Organic Carbon

Treatments	pH	EC (dSm^{-1})	TOC (g/100cc)
Lcs_{40}	5.56 b	25.9 b	1.60 ab
Lcs_{80}	5.54 b	25.87 b	1.47 bc
Lfc_{40}	6.26 a	11.08 c	1.29 d
Lfc_{80}	6.35 a	11.3 c	1.44 c
Lsb_{40}	6.24 a	26.7 ab	1.35 cd
Lsb_{80}	5.59 b	25.9 b	1.59 ab
L	6.31 a	27.9 a	1.65 a

Means within a column followed by the same letter are not significantly different at p = o.o5

Lcs=leachate + copper sulfate, Lfc=leachate+ ferric chloride, Lsb=leachate+ sodium benzoate, L=control (pure leachate)

Table 3 showed that all chemical salts reduced total number of microorganisms, bacteria, coli forms and fungi when compared to pure MSW leachate. Total number of microorganisms in ferric chloride and sodium benzoate treatments was reduced significantly (-40 and -43.8% respectively) when compared to copper sulfate treatment .Though, number of bacteria in both treatments was reduced significantly (-43.5 and -41.8%), when compared to copper sulfate treatment (Table 3).

Number of coli forms (fecal and non fecal) in copper sulfate (-44%) and sodium benzoate (-42%) treatments showed significant reductions when compared to ferric chloride treatment (Table 3).

Table 3

Effect of type of chemical salts on total number of microorganisms, bacteria, coli forms and fungi

Treatments	Total No. of Microorganisms (1×10^5)/ml	No. of Bacteria (1×10^5)/ml	No. of Coli forms (Fecal and Non Fecal) (1×10^2)/ml	No. of Fungi (1×10^3)/ml
Lcs	1.895 b	1.778 b	2.425 c	11.43 c
Lfc	1.138 c	1.005 c	4.350 b	14.32 b
Lsb	1.065 c	1.035 c	2.575 c	3.975 d
L	14.204 a	10.878 a	30.33 a	29.3 a

Means within a column followed by the same letter are not significantly different at p = o.o5

Lcs=leachate + copper sulfate, Lfc=leachate+ ferric chloride, Lsb=leachate+ sodium benzoate, L=control (pure leachate)

Number of fungi in sodium benzoate (-72%) and copper sulfate (-20%) treatments significant reductions compared to ferric chloride treatment (Table3).

Effect of 40 and 80 mg l^{-1} salts on microorganisms (table 4) showed that total number of microorganisms, number of bacteria and fungi in 40 mg l^{-1} salt had -92.8, -91.2 and -75.06% significant reduction respectively when compared to pure MSL leachate.

Results indicated that 40 mg l^{-1} amount of salt compared to 80 mg l^{-1} had better effect on the total amount of microorganisms (-40%), number of bacteria (-40.4%) and number of fungi (-41.6%). Maximum reduction (-99.93%) in number of coli forms (fecal and non fecal) was observed in 80 mg l^{-1} of salt.

Table 4

Effect of amount of chemical salt on total number of microorganisms, bacteria, coli forms and fungi

Treatments (mg l^{-1})	Total No. of Microorganisms (1×10^5)/ml	No. of Bacteria (1×10^5)/ml	No. of Coli forms (Fecal and Non Fecal) (1×10^2)/ml	No. of Fungi (1×10^3)/ml
40	1.021 b	0.951 b	4.383 a	7.305 b
80	1.711 a	1.595 a	1.85 b	12.511 a

Means within a column followed by the same letter are not significantly different at p = o.o5

Fig. 1 showed that maximum significant reduction on total number of microorganisms noted in 40 mg l^{-1} ferric chloride, followed by 40 and 80 mg l^{-1} sodium benzoate treatments when compared to other treatments

.

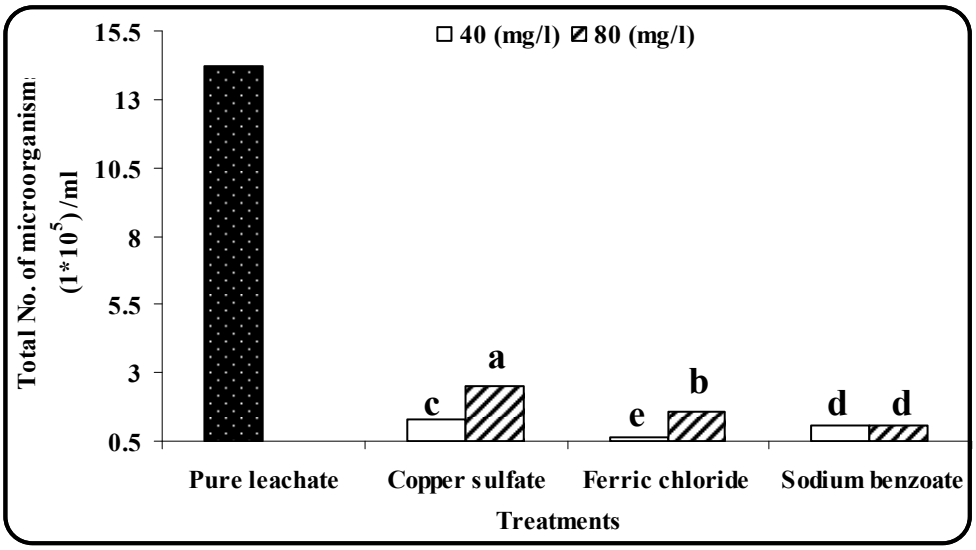

Figure 1

Type and amount of chemical salts' effects on number of total microorganisms

Maximum significant reduction in number of bacteria was recorded in 40 mg l^{-1} ferric chloride, followed by 40 and 80mg l^{-1} sodium benzoate treatments (Fig. 2).

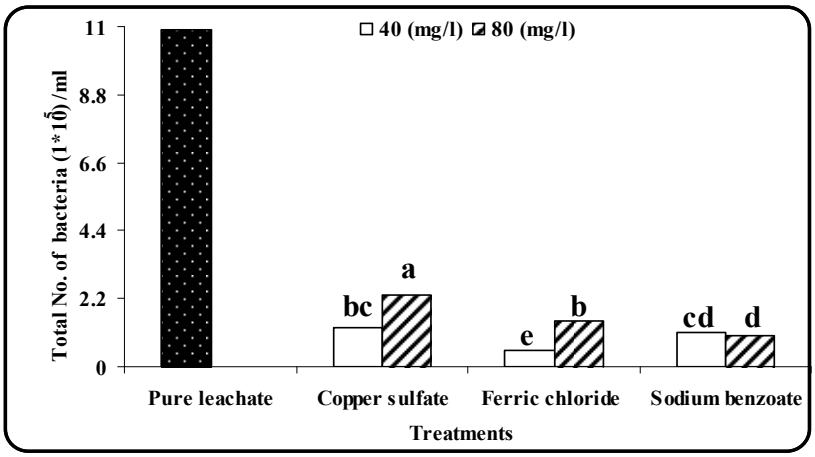

Fig 2
Type and amount of chemical salts' effects on number of bacteria

Maximum significant reduction in number of coli forms in treated leachate was recorded in 80 mg l^{-1} sodium benzoate, followed by 40 mg l^{-1} copper sulfate and 80 mg l^{-1} ferric chloride treatments compared to other treatments (Fig 3).

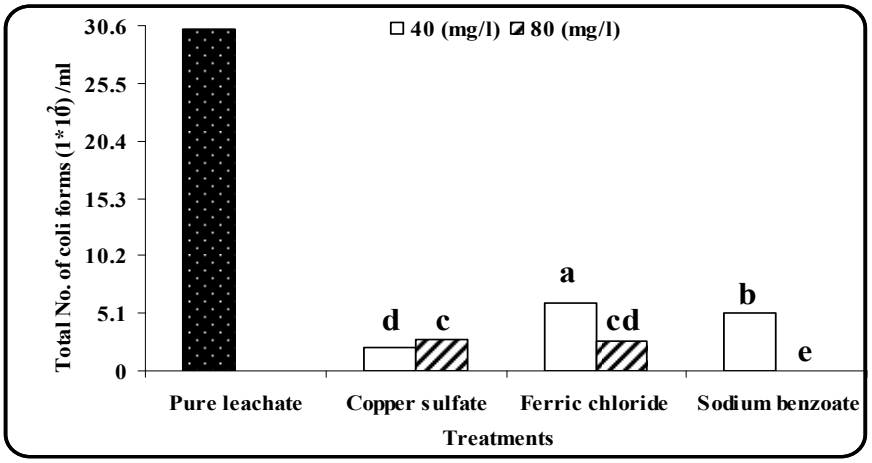

Fig 3
Type and amount of chemical salts' effects on number of coli forms (fecal and non fecal)

Maximum reduction in number of fungi (Fig 4) was recorded in 40 mg l^{-1} sodium benzoate, followed by 40 mg l^{-1} copper sulfate and 80 mg l^{-1} sodium benzoate treatment, respectively.

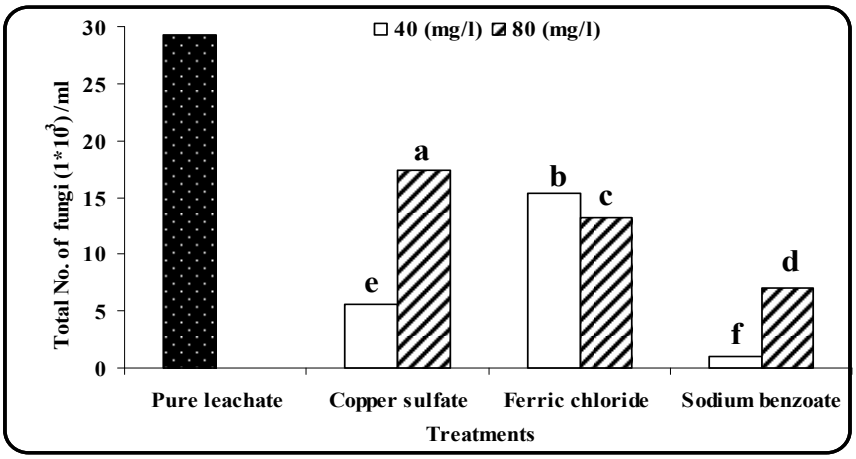

Fig 4
Type and amount of chemical salts' effects on number of fungi

Identification of microorganisms (bacteria, fungi and coli forms (fecal and non fecal) in all treatments showed that use of these three coagulants in both amount (40 and 80 mg l^{-1}) reduced number of *Staphylococcus*, *Streptococcus*, *Bacillus* and *Streptomycete* bacteria, yeasts, *Aspergillus niger*, *Aspergillus fumigatus*, *Geotrrikum*, *Penicillium* and *Fusarium* fungi .Number of fecal and non fecal coli forms were reduced significantly .

DISCUSSION

Addition of copper sulphate (40, 80 mg l^{-1}) and sodium benzoate (80 mg l^{-1}) reduced significantly the pH of MSW leachate. This may be due to higher acidity nature of sulfate salt than chloride salt.

Electrical conductivity of MSW leachate treated with chemical salts except 40 mg l^{-1} sodium benzoate reduced significantly when compared to pure lechate (Table 2). Since the amount of total soluble salts (salinity) affects the plants and animals surviving via osmotic process [13], electrical conductivity of MSW leachate with the use of these compounds and salts due to coagulation process decreased. The comparison of alum and ferric chloride showed that ferric chloride had better effect on coagulation process [2].

The soluble organic carbon of all treatments under the effect of chemical salts reduced when compared to pure lechate (Table 2). Maximum reduction in soluble organic carbon of MSW leachate noted in ferric chloride (-23%) and sodium benzoate (-18%), having 40 mg l^{-1} concentration which is similar with the results of Steven et al.[4].

All chemical salts caused significant reduction in the total number of microorganisms when compared to pure MSW leachate. It appears that, the increasing of coagulation process resulted in the reduction of total microorganism numbers [2,14].

Marthakutty et al. [5] and Randan [15] confirmed antibacterial and antifungal affects of copper complexes with Cl^-, Br^-, $NO3^-$, NCS^-, N_3^- and SO_4^{-2} ligands. They concluded that these effects in copper complexes with these ions are much stronger than their free ligands.

Also, our results showed that sodium benzoate compared of other salts had much better effect on total number of microorganism's reduction. Ogiehor et al. [6] confirmed that no aflatoxin was observed in sodium benzoate treatments. Lopez-Malo et al. [7] showed similar results with sodium benzoate salt on fungi too.

Results of this study showed that (Table 4) 40 mg l^{-1} of salt have a better effect on microbial population reduction than 80 mg l^{-1}. High concentration of sulfate in MSW leachate affects the pH of solution for coagulation process. So, salt concentrations of 80 mg l^{-1} or more produced a drastic reduction in coagulation due to pH alteration. According to Keshavarze et al. [13], 125 mg l^{-1} sulfate ion used, reduced pH of solution from 6.65 to 6.0 and thereby the salt efficiency for coagulation reduced.

In addition, the use of chemical salts on microbial detoxification of MSW leachate showed that ferric chloride (40 mg l^{-1}) and sodium benzoate (40, 80 mg l^{-1}) had maximum reducing impact on total microorganisms: bacteria and fungi number (Fig. 1, 2 and 3).

Mills et al. [8] confirmed that sodium salt was better on mycelial growth and spore germination when compared to other salts of various potato pathogens. Spore germination in most pathogens such as *Alternaria altrnata*, *Botrytis cinerea*, *Fuusarium solani* var. was consistently inhibited by the aluminum compounds (aluminum chloride, aluminum acetate and alum) and the commercial fungicides: mancozeb and copper sulfate.

Copper sulfate salt of 40 mg l^{-1} next to ferric chloride and sodium benzoate showed a reducing effect on total number of microorganisms, number of bacteria and fungi. Frank and Hasman [16] on susceptibility of different bacterial species such as *Salmonella* and *E. coli* and… to copper sulfate and zinc chloride and antimicrobial substances concluded that there were large differences in the intrinsic susceptibility of the different bacterial species to these compounds and *Staphylocococi* were in general very susceptible to all antimicrobial compounds tested But, *Salmonella* was in general less susceptible to copper sulfate and other compounds, followed by *E. coli* and the Gram-positive species. *Salmonella* especially seemed intrinsically less susceptible than the other bacterial species, which might have human health implications. Also, *Enterococci* was the only species resistant to copper sulfate in Frank and Hasman [16] research. The number of focal and non focal coli forms reduced in copper sulfate compared of other salts. Generated silver and copper ions with chlorine in cooling systems decreased 99% of microbial population ,total number of coli forms ,sulfate reducing bacteria and slime-forming bacteria ,whereas, treatment only with chlorine decreased 90% of microbial population [17].

Zacheus et al. [18] showed that manganese and copper in the deposits correlated negatively with the numbers of heterotrophic bacteria. Maximum reduction in total microorganisms and bacteria numbers were observed in MSW leachate + sodium benzoate and also MSW leachate + ferric chloride treatments, while the maximum reduction in number of fungi was observed in MSW leachate + sodium benzoate. The number of focal and non focal coli forms reduced significantly in MSW leachate + copper sulfate and MSW leachate + sodium benzoate. Therefore, the use of sodium benzoate and copper sulfate salts is recommended**.** Since maximum deleterious effects with respect to human and animal health are related to coli forms especially fecal coli forms and the number of fungi is important in plant disease. Maximum significant reduction in total microorganisms, bacterial and fungi number observed at 40 mg l^{-1} salt, whereas, with respect to fecal and non fecal coli forms, number, 80 mg l^{-1} salt concentration is needed.

REFERENCES

1. Kent DK, Water Treatment Plant Operation. Vol 1 Chapter 4.Coagulation and

Flocculation, California State University, Sacramento, School of Engineering, USA: 141-144(1992).
2. Ghadbanan Sh, And M Amiri, Comparison of alum and ferric chloride coagulants working to reduction of sulphate paper production waste water, Water and Waste Water, 44:55-60 (2002).
3. Montogomery, JM, Water Treatment Principles and Design, John Wiley and Sons Inc, USA (1995).
4. Steven K, Gossett DM, and JM Gossett, Effect of chemical coagulation on anaerobic digestibility of organic materials, Water Research, 16(5):707-718(2003).
5. Marthakutty J, Suni V, Maliyeckal R, Kurup P, Nethaji M, Kishore A, and SG Bhat, Structural, spectral and antimicrobial studies of copper (II) complexes of 2-benzoylpyridine N(4)-cyclohexyle thiosemicarbazone, Polyhedron, 23:3069-3080(2004).
6. Ogiehor IS, and MJ Ikenebomeh, Antimicrobial effects of sodium benzoate on the growth, survival and aflatoxin production of some species of Aspergillus in garri during storage, Pakistan Journal of Nutrition, 3(5):300-303(2004).
7. Lopez-Malo A, Alzamora SM, and E Palou, Aspergillus flavus growth in the presence of chemical preservatives and naturally occurring antimicrobial compounds, International Journal of Food of Microbiology, 99:119 – 128(2005).
8. Millis A AS, Platt HW, and RA R Hurta, Effect of salt compounds on mycelia growth, sporulation and spore germination of various potato pathogens, Post harvest Biology and Technology, 34:341-350(2004).
9. Walkley A, and IA Black, An examination of Degtjareff method for determining organic carbon in soils: effect of variations in digestion conditions and of inorganic soil constituents, Soil Science, 63: 251–263(1934).
10. Frazzier WC, Food Microbiology, McGraw-Hill, Inc, Second Edition, Printed in the United States of America, P: 293-302(1967).
11. Mannion JB, and BD Haskins, Introduction to Water Quality Analyses, American Water Works Association, 4:273-282(1982).
12. Tershak MJ, Microbiology 202, Supplementary Information, Coordinator, Microbiology 202, 119S Frear 863-2638(1992) .
13. Keshavarze Shokri A, Sheydani M, and SH Mohamad Rezayee Omran, Water Contamination, Laboratory Experiment of Chemical, Physical and Biological Factors, Siyavash Print(1998).
14. Shahmansouri M, and A Neshat, Comparison of poly aluminum chloride, aluminum sulfate and ferric chloride in removal of TOC and total coli form, Water and Waste Water, 48:30-44(2003).
15. Ramadan AM, Structural and biological aspects of copper (II) complexes with2-methyl-3-amino-(3H)-quinazolin-4-one.Journal of Inorganic Biochemistry, 65:183-189(1997).
16. Frank MA, and H Hasman, Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection, Veterinary Microbiology, 100:83 – 89(2004).
17. Martinez SS, Gallegos AA, and E Martinez, Electrolytically generated silver and copper ions to treat cooling water :an environmentally friendly novel alternative .International Journal of Hydrogen Energy, 29:921-932(2004).
18. Zacheus OM, Lehtola MJ, Korhonen LK, and PJ, Martikainen ,Soft deposits, the key site for microbial growth in drinking water distribution networks, Water Research, 35(7):1757 – 1765(2001).