Editorial Team

JAEHR
Journal of Advances in Environmental Health Research
Environmental Health Research Center
دارای امتیاز علمی- پژوهشی از کمیسیون نشریات علوم پزشکی کشور
Kurdistan University of Medical Sciences
eISSN: 2345-3990

Information for Authors

AIM AND SCOPE

*Journal of Advances in Environmental Health Research (JAEHR)*is a quarterly peer-reviewed scientific journal published by Kurdistan University of Medical Sciences. The manuscripts on the topic of environmental science and engineering will be published in this journal. This contains all aspects of solid waste management, air pollution, water and wastewater, environmental monitoring and modeling, innovative technologies and studies related to the environmental science.

Instruction to Authors

MANUSCRIPTS

Manuscripts containing original material are accepted for consideration if neither the article nor any part of its essential substance, tables, or figures has been or will be published or submitted elsewhere before appearing in the *Journal of Advances in Environmental Health Research*. This restriction does not apply to abstracts or press reports published in connection with scientific meetings. Copies of any closely related manuscripts must be submitted along with the manuscript that is to be considered by the *Journal of Advances in Environmental Health Research*. Authors of all types of articles should follow the general instructions given below.

HUMAN AND ANIMAL RIGHTS

The research involves human beings or animals must adhere to the principles of the Declaration of Helsinki (http://www.wma.net/e/ethicsunit/helsinki.htm).

Types of Articles

- *Original article* which reports the results of an original scientific research should be less than 4000 words.
- *Review article* which represents the researches and works on a particular topic.
- *Brief communication* is a short research article and should be limited to 1500 words. This article contains all sections of an original article.
- *Case report* is a detailed report of an individual patient that may represent a previously non-described condition and contains new information about different aspects of a disease. It should be less than 2000 words.
- *Letter to the Editor* must be less than 400 words in all cases.
- *Book Review* must be less than 1000 words on any book topics related to the scope of *Journal of Advances in Environmental Health Research*.

SUBMISSION

- Only online submission is acceptable. Please submit online at: http://www.jaehr.muk.ac.ir
- This manuscripts should be divided into the following sections: (1) Title page, (2) Abstract and Keywords, (3) Introduction, (4) Materials and Methods, (5) Results and Discussion, (6) Acknowledgements, (7) Author contribution, (8) References, (9) Figure legends, (10) Tables and (11) Figures (figures should be submitted in separate files if the file size exceeds 2 Mb).
- Please supply a word count in title page.
- Use normal page margins (2.5 cm), and double-space throughout the manuscript.
- Use Times New Roman (12) font throughout the manuscript.
- Prepare your manuscript text using a Word processing package (save in .doc or .rtf format). Submissions of text in the form of PDF files are not permitted.

COVER LETTER

A covering letter signed by all authors should identify the corresponding author (include the address, telephone number, fax number, and e-mail address). Please make clear that the final manuscript has been seen and approved by all authors, and that the authors accept full responsibility for the design and conduct of the study, had access to the data, and controlled the decision to publish.
Authors are also asked to provide the names and contact information for three potential reviewers in their cover letter. However, the journal is not obliged to use the suggested reviewers. Final selection of reviewers will be determined by the editors.

AUTHORSHIP

As stated in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org/icmje-recommendations.pdf), credit for authorship requires substantial contributions to: 1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND 2. Drafting the work or revising it critically for important intellectual content; AND 3. Final approval of the version to be published; AND 4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Each author must sign authorship form attesting that he or she fulfills the authorship criteria. There should be a statement in manuscript explaining contribution of each author to the work. Acknowledgments will be limited to one page of *Journal of Advances in Environmental Health Research*, and those acknowledged will be listed only once.

Any change in authorship after submission must be approved in writing by all authors.

ASSURANCES

In appropriate places in the manuscript please provide the following items:

- If applicable, a statement that the research protocol was approved by the relevant institutional review boards or ethics committees and that all human participants gave written informed consent
- The source of funding for the study
- The identity of those who analyzed the data
- Financial disclosure, or a statement that none is necessary

TITLE PAGE

With the manuscript, provide a page giving the title of the paper; titles should be concise and descriptive (not declarative). Title page should include an abbreviated running title of 40 characters, the names of the authors, including the complete first names, the name of the department and institution in which the work was done, the institutional affiliation of each author. The name, post address, telephone number, fax number, and e-mail address of the corresponding author should be separately addressed. Any grant support that requires acknowledgment should be mentioned on this page. Word count of abstract and main text as well as number of tables and figures and references should be mentioned on title page. If the work was derived from a project or dissertation, its code should also be stated.

Affiliation model: Department, Institute, City, Country.
Example: Department of Environmental Health Engineering, School of Health, Kurdistan University of Medical Sciences, Sanandaj, Iran.

ABSTRACT

Provide on a separate page an abstract of not more than 250 words. This abstract should consist of ONE paragraph (Non-structured Abstract). It should briefly describe the problem being addressed in the study, how the study was performed, the salient results, and what the authors conclude from the results respectively. Three to seven keywords may be included. Keywords are preferred to be in accordance with MeSH (http://www.ncbi.nlm.nih.gov/mesh) terms.

CONFLICT OF INTEREST

Authors of research articles should disclose at the time of submission any financial arrangement they may have with a company whose product is pertinent to the submitted manuscript or with a company making a competing product. Such information will be held in confidence while the paper is under review and will not influence the editorial decision, but if the article is accepted for publication, a disclosure will appear with the article.
Because the essence of reviews and editorials is selection and interpretation of the literature, the *Journal of Advances in Environmental Health Research* expects that authors of such articles will not have any significant financial interest in a company (or its competitor) that makes a product discussed in the article.

REVIEW AND ACTION

Submitted papers will be examined for the evidence of plagiarism using some automated plagiarism detection service. Manuscripts are examined by members of the editorial staff, and two thirds are sent to external reviewers. Communications about manuscripts will be sent after the review and editorial decision-making process is complete within **3-6 weeks** after receiving the manuscript. After acceptance, editorial system makes a final language and scientific edition. No substantial change is permitted by authors after acceptance. It is the responsibility of corresponding author to answer probable questions and approve final version.

COPYRIGHT

Journal of Advances in Environmental Health Research is the owner of all copyright to any original work published by the

JAEHR. Authors agree to execute copyright transfer forms as requested with respect to their *Journal of Advances in Environmental Health Research* has the right to use, reproduce, transmit, derive works from, publish, and distribute the contribution, in the *Journal* or otherwise, in any form or medium. Authors will not use or authorize the use of the contribution without the Journal Office' written consent

JOURNAL STYLE

Tables

Double-space tables and provide a title for each.

Figures

Figures should be no larger than 125 (height) x 180 (width) mm (5 x 7 inches) and should be submitted in a separate file from that of the manuscript. The name of images or figures files should be the same as the order that was used in manuscript (fig1, fig2, etc.). Only JPEG, tif, gif and eps image formats are acceptable with CMYK model for colored image at a resolution of at least 300 dpi. Graphs must have the minimum quality: clear text, proportionate, not 3 dimensional and without disharmonic language. Electron photomicrographs should have internal scale markers.

If photographs of patients are used, either the subjects should not be identifiable or the photographs should be accompanied by written permission to use them. Permission forms are available from the Editorial Office.

Scientific illustrations will be created or recreated in-house. If an outside illustrator creates the figure, the *Journal of Advances in Environmental Health Research* reserves the right to modify or redraw it to meet our specifications for publication. The author must explicitly acquire all rights to the illustration from the artist in order for us to publish the illustration. Legends for figures should be an editable text as caption and should not appear on the figures.

References

The Vancouver style of referencing should be used. References must be double-spaced and numbered as **superscripts** consecutively as they are cited. References first cited in a table or figure legend should be numbered so that they will be in sequence with references cited in the text at the point where the table or figure is first mentioned. List all authors when there are six or fewer; when there are seven or more, list the first six, then "et al." The following are sample references:

1. Maleki A, Shahmoradi B, Daraei H, Kalantar E. Assessment of ultrasound irradiation on inactivation of gram negative and positive bacteria isolated from hospital in aqueous solution. J Adv Environ Health Res 2013; 1(1): 9-14.

2. Buckwalter JA, Marsh JL, Brown T, Amendola A, Martin JA. Articular cartilage injury. In: Robert L, Robert L, Joseph V, editors. Principles of Tissue Engineering. 3rd ed. Burlington, MA: Academic Press; 2007. p. 897-907.

3. Kuczmarski RJ, Ogden CL, Grammer-Strawn LM, Flegal KM, Guo SS, Wei R, et al. CDC growth charts: United States. Advance data from vital and health statistics. No. 314. Hyattsville, Md: National Center for Health Statistics, 2000. (DHHS publication no. (PHS) 2000-1250 0-0431)

4. World Health organization. Strategic directions for strengthening nursing and midwifery services [online]. Available from: URL:http://www.wpro.who.int/themesfocuses/theme3/focus2/nursingmidwifery.pdf2002

Units of Measurement

Authors should express all measurements in conventional units, with Système International (SI) units given in parentheses throughout the text. Figures and tables should use conventional units, with conversion factors given in legends or footnotes. In accordance with the Uniform Requirements, however, manuscripts containing only SI units will not be returned for that reason.

Abbreviations

Except for units of measurement, abbreviations are discouraged. Consult Scientific Style and Format: The CBE Manual for Authors, Editors, and Publishers (Sixth edition. New York: Cambridge University Press, 1994) for lists of standard abbreviations. Except for units of measurement, the first time an abbreviation appears, it should be preceded by the words for which it stands.

Chemical Structure

Structures should be produced with a chemical drawing program, preferably ChemDraw 4.5 or higher, and submitted in TIFF format to allow use of electronic files in production. Structures should also be submitted in native file formats, e.g., RDX.

For any more detail about the writing style for your manuscripts refer to:

http://www.jaehr.muk.ac.ir

Authorship Form

Title of the manuscript:

.....

.....

We, the undersigned, certify that we take responsibility for the conduct of this study and for the analysis and interpretation of the data. We wrote this manuscript and are responsible for the decisions about it. Each of us meets the definition of an author as stated by the International Committee of Medical Journal Editors (see http://www.icmje.org/icmje-recommendations.pdf). We have seen and approved the final manuscript. Neither the article nor any essential part of it, including tables and figures, will be published or submitted elsewhere before appearing in the *Journal of Advances in Environmental Health Research* [All authors must sign this form or an equivalent letter.]

Name of Author	**Contribution**	**Signature**

Please scan this form and upload it as a supplementary file in "Step 4" of submitting articles.

Table of Contents

Original Article(s)

Journal of Advances in Environmental Health Research

Effect of waterborne copper oxide nanoparticles and copper ions on guppy (Poecilia reticulata): Bioaccumulation and histopathology

Borhan Mansouri[1], Raouf Rahmani[2], Nammam Ali Azadi[3], Behrouz Davari[4], Seyed Ali Johari[5], Pedram Sobhani[5]

1 Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran
2 Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
3 Department of Biostatistics, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
4 Department of Medical Entomology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
5 Department of Fisheries, School of Natural Resources, University of Kurdistan, Sanandaj, Iran

Original Article

Abstract

The aim of the present study was to investigate the effect of copper oxide nanoparticles (CuO-NPs) and copper ions (Cu^{++}) on guppy (Poecilia reticulata), in order to assess Cu uptake in the gill, and histopathology of gill and intestinal organs in semi-static regimes for 10 days. Guppy fish were assigned into three groups; one control group, and two experimental groups receiving 20 µg/l of either Cu^{++} or CuO-NPs in a semi-static aqueous culture for 10 days. Gill and intestinal tissue samples were obtained under a standard protocol for further histopathological examinations. The notable alterations observed in gill tissues in the experimental groups were aneurism, fusion, gill epithelial hyperplasia, increased mucous secretion, and necrosis. Noticeable anomalies in intestinal tissue were increase in the number of goblet cells, swelling of goblet cells, degeneration, vacuolation, necrosis, and erosion. Moreover, copper accumulation in gill tissue in the Cu^{++} treated group was higher than that in the CuO-NPs treated group. In contrast, the severity of histopathological damages in gill and intestinal tissues was greater in the CuO-NPs experimental group.

KEYWORDS: Gills, Goblet cells, Hyperplasia, Nanoparticles, Copper

Date of submission: 16 May 2015, ***Date of acceptance:*** 19 Jul 2015

Citation: Mansouri B, Rahmani R, Azadi NA, Davari B, Johari SA, Sobhani P. **Effect of waterborne copper oxide nanoparticles and copper ions on guppy (Poecilia reticulata): Bioaccumulation and histopathology.** J Adv Environ Health Res 2015; 3(4): 215-23.

Introduction

Copper (Cu) in low amounts is considered as an essential micronutrient to all living organisms because it acts as a cofactor for various enzymes responsible for performing essential metabolic activity.[1] However, excessive concentrations of copper in aquatic ecosystems can exert adverse toxicological effects on freshwater organisms such as fish.[2] In the last decade, several studies reported that waterborne exposure to soluble Cu can induce endocrine disruption and affect metabolic rates,[3,4] oxidation stress, cell apoptosis, immune responses,[5] swimming behavior,[6] histopathology,[4,7] growth parameters, digestive enzymes, and body composition.[7] In recent decades, copper oxide nanoparticles (CuO-NPs) have found a wide spectrum of applications such as gas sensors,[8] catalytic processes,[9] solar cells and lithium batteries,[10] face masks, wound dressings, and socks.[11,12] There is a growing concern that these products and their byproducts may

Corresponding Author:
Borhan Mansouri
Email: borhanmansouri@yahoo.com

discharge hazardous biochemical particles into aquatic habitats which in turn can affect their biota.

Several studies have been conducted on the toxicity of copper and copper oxide nanoparticles to aquatic organisms, and these studies have shown that these substances can be toxic to aquatic organisms such as fish.[1,3,5,7] Wang et al. reported the toxic effect of either Cu-NPs or $CuSO_4$ exposure on juvenile Epinephelus coioides.[5] They found that either form of Cu exposure inhibited in digestive enzyme activities contribute to the diminished growth performance of Epinephelus coioides. In another study, Abdel-Khalek et al. studied the toxic effects of CuO [bulk particles (BPs) and nanoparticles (NPs)] at various concentrations and concluded that it can induce biochemical alterations and oxidative stress in Nile tilapia (Oreochromis niloticus).[13] Moreover, Shaw et al. showed significant Cu accumulation in gill over time after treating fish with Cu and $CuSO_4$ treatments.[14] They reported similar toxic effects for Cu-NPs and $CuSO_4$.

Fish are frequently used in toxicological studies as a biological indicator. Fish are sensitive to many variables in their environment; hence, they play a significant role in assessment of water quality.[15] In this regard, due to its unique characteristics, gill tissue is used routinely in aquatic toxicology studies. This tissue becomes the first target of waterborne NPs due to its direct contact with the external environment and the large surface area of gill exposed to the pollutants, as well as the main place for copper uptake.[16-19] Therefore, our study purposed to further investigate the effects of copper oxide nanoparticles (CuO-NPs) and copper ions (Cu^{++}) on guppy (Poecilia reticulata) to compare CuO-NPs and Cu^{++} bioaccumulation in the gill, and determine the histopathology of intestine and gill of guppy.

Materials and Methods

The CuO-NPs (CuO purity: 99+٪, 40 nm) used in this study were produced by US Research Nanomaterials, Inc. (3302 Twig Leaflane, Houston, TX77084) and purchased from Nanosany Co. (Mashhad, Iran). The purity, and morphology and mean unaggregated particle diameters of CuO-NPs were determined through transmission electron microscopy (TEM) and scanning electron microscopy (SEM), respectively (Figure 1). The other characteristics of CuO-NPs were 20 m^2/g specific surface area (SSA), 6.4 g/cm true density, and 0.79 g/cm bulk density. Moreover, the copper ions used in the form of cupric sulphate ($CuSO_4$ $5H_2O$) were produced by BDH Chemical Ltd Poole, England.

Guppy (Poecilia reticulata) with a mean total length of 3 ± 0.4 cm and mean weight of 2 ± 0.4 g were obtained from a local aquaculture shop in Sanandaj, Iran. Prior to the beginning of experiments, guppy fish were acclimatized in 50 l tanks supplied with continuously aerated tap water (22-27 ℃) under 12-hour light/12-hour dark cycles for a photoperiod of one month. Fish were fed with commercially available fish food (Tetra) at a rate of 2% body weight per day. The characteristics of the water used for the guppy were 7.3 ± 0.3 pH, 600 ± 10 µS/cm conductivity, 5° degrees of general hardness (dGH), 25.0 ± 1 °C temperature, and 6.2 ± 0.6 mg/l dissolved oxygen content (DO).

For our evaluation, 20 µg/l of either CuO-NPs or $CuSO_4.5H_2O$ was used to reflect the actual environmental concentration.[14] Fish were divided into three groups. One group served as control, and the other two groups were treated with CuO-NPs and Cu^{++}, respectively. Within each group, three fish were randomly selected for further studies. Using a 12 l aquarium, the effect of the interventions was assessed in two periods of exposure (5 and 10 days). To minimize degradation of CuO-NPs and Cu^{++} concentrations, half of the water in the aquariums was renewed every day. Moreover, during the exposure, the tanks were aerated to prevent the propensity of aggregation. At the end of the experiment,

bioaccumulation and histopathology studies were carried out for selected specimens.

For the evaluation of bioaccumulation, on day 5 and 10, dissections were performed to isolate gill organs (2 fishes were pooled). Gill samples were digested in a solution of nitric acid (HNO_3) and perchloric acid ($HClO_4$). Samples were accurately weighed and separated into 50-ml Erlenmeyer flasks and 5 ml nitric acid (65%) was added to each sample. Before adding 2.5 ml perchloric acid (70%) to each simple, they were left out overnight to be slowly digested. Digestion was performed on the bain-marie (water bath) at 100 ^{o}C until the solutions were cleared. Then, the digested samples were diluted with 25 ml deionized water.[20,21] Finally, the concentration of Cu was measured using a Phoenix 886 flame furnace atomic absorption spectrophotometer.

For the histopathology evaluation of each experimental group and control group, 4 fish were sacrificed to remove their gills. Gills were fixed in Bouin solution and their tissues were dehydrated using a series of graded ethanol solutions and were cleared in xylene. Samples were embedded in paraffin wax and portions of 5 µm were prepared from paraffin blocks using a rotary microtome. These portions were then stained with haematoxylin-eosin and examined microscopically.[22] The diameter and length of secondary gill lamellas as well as diameter of gill filaments were measured using the Axio Vision software (Release 4.8.2, Zeiss, Germany).

The SPSS software (version 16, SPSzA S Inc., Chicago, IL, USA) was used for data analysis. To compare the mean of copper concentration accumulated in the gill tissues between treatment groups one-way analysis of variance (ANOVA) was applied. Data were log transformed to meet the homogeneity of variance required by ANOVA. Ethical principles and animal rights were applied in this research and the study was approved by the Ethics Committee of the Kurdistan University of Medical Sciences, Iran (MUK.REC.1393.98).

For our evaluation, 20 µg/l of either CuO-NPs or $CuSO_4.5H_2O$ was used to reflect the actual environmental concentration.[14] Fish were divided into three groups. One group served as control, and the other two groups were treated with CuO-NPs and Cu^{++}, respectively. Within each group, three fish were randomly selected for further studies. Using a 12 l aquarium, the effect of the interventions was assessed in two periods of exposure (5 and 10 days). To minimize degradation of CuO-NPs and Cu^{++} concentrations, half of the water in the aquariums was renewed every day. Moreover, during the exposure, the tanks were aerated to prevent the propensity of aggregation.

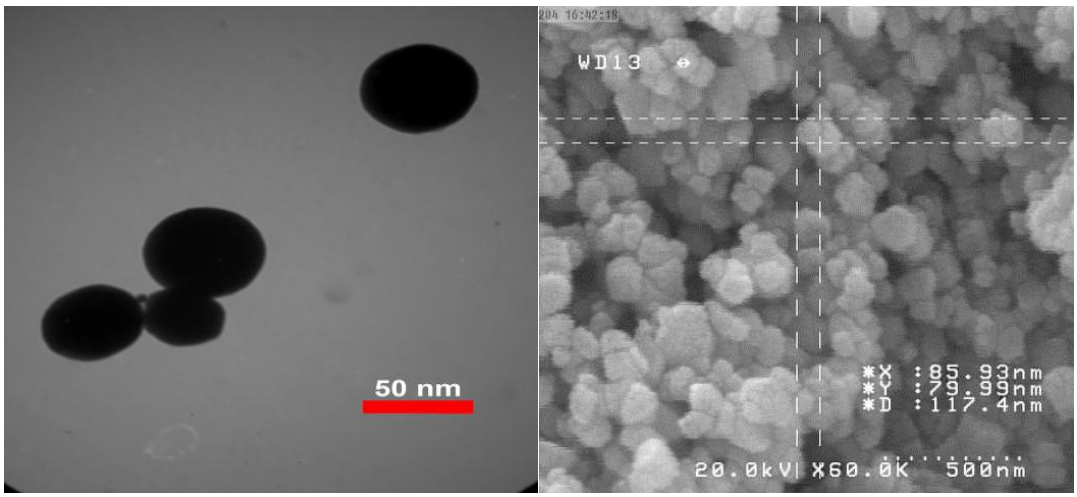

Figure 1. Transmission electron microscopy (TEM) (left) and scanning electron microscopy (SEM) (right) images of tested CuO nanoparticles

At the end of the experiment, bioaccumulation and histopathology studies were carried out for selected specimens.

For the evaluation of bioaccumulation, on day 5 and 10, dissections were performed to isolate gill organs (2 fishes were pooled). Gill samples were digested in a solution of nitric acid (HNO_3) and perchloric acid ($HClO_4$). Samples were accurately weighed and separated into 50-ml Erlenmeyer flasks and 5 ml nitric acid (65%) was added to each sample. Before adding 2.5 ml perchloric acid (70%) to each simple, they were left out overnight to be slowly digested. Digestion was performed on the bain-marie (water bath) at 100 ^{o}C until the solutions were cleared. Then, the digested samples were diluted with 25 ml deionized water.[20,21] Finally, the concentration of Cu was measured using a Phoenix 886 flame furnace atomic absorption spectrophotometer.

For the histopathology evaluation of each experimental group and control group, 4 fish were sacrificed to remove their gills. Gills were fixed in Bouin solution and their tissues were dehydrated using a series of graded ethanol solutions and were cleared in xylene. Samples were embedded in paraffin wax and portions of 5 µm were prepared from paraffin blocks using a rotary microtome. These portions were then stained with haematoxylin-eosin and examined microscopically.[22] The diameter and length of secondary gill lamellas as well as diameter of gill filaments were measured using the Axio Vision software.

The SPSS software (version 16, SPSS Inc., Chicago, IL, USA) was used for data analysis. To compare the mean of copper concentration accumulated in the gill tissues between treatment groups one-way analysis of variance (ANOVA) was applied. Data were log transformed to meet the homogeneity of variance required by ANOVA. Ethical principles and animal rights were applied in this research and the study was approved by the Ethics Committee of the Kurdistan University of Medical Sciences, Iran (MUK.REC.1393.98).

Results and Discussion

For treatment groups, the accumulated copper in the gills of fish samples are given in table 1. Results are shown in the form of means ± standard deviation (SD). It appears that the Cu uptake was higher in the group treated with Cu^{++} than the CuO-NPs group, but the difference was not statistically significant at nominal 5% level (one-way ANOVA, $P > 0.05$). However, the accumulated copper in the gills of fish treated with either Cu^{++} or CuO-NPs were significantly higher than that observed in the control group (one-way ANOVA, $P < 0.05$).

Table 1. Copper accumulation in the gill of guppy following 5 or 10 days exposure to 20 µg/l of CuO-NPs and Cu^{++}

Tissue	Gill	
Groups	**5 days**	**10 days**
CuO-NPs	0.93 ± 0.01*	1.45 ± 0.11*
Cu^{++}	1.11 ± 0.13*	1.53 ± 0.12*
Control	0.24 ± 0.07**	0.20 ± 0.04**
P**	0.05	0.05

* In each column, the numbers with different letters differ significantly ($P > 0.05$); ** One-way ANOVA

The predominant gill responses to CuO-NPs and Cu^{++} exposure were an aneurism, fusion, gill epithelial hyperplasia, increased mucous secretion, and necrosis (Figure 2). Anomalies were also observed in the intestinal organs including increase in the number of goblet cells, swelling of goblet cells, degeneration, vacuolation, necrosis, and erosion (Figure 3). The results presented in tables 2 and 3 illustrate that the severity of damages to the gill (manifesting as fusion and necrosis) and intestine (manifesting as degeneration and necrosis) in the CuO-NPs treatment group was greater than those in the Cu^{++} group.

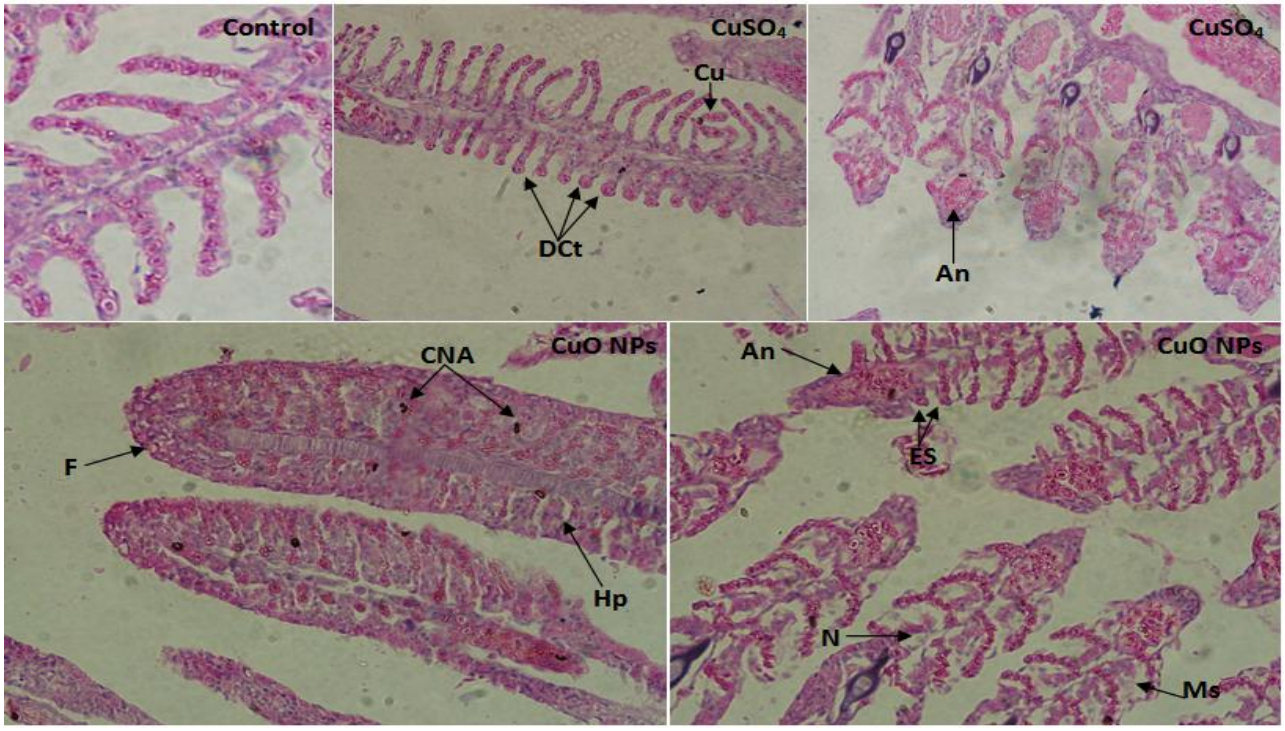

Figure 2. Histological alterations in the gills of guppy after 10 days of exposure to CuO-NPs and Cu^{++} (x40)
Gills of fish in the control group showed only some minor histopathological alterations, whereas treatment groups showed injuries including aneurism (An), dilated and clubbed tips (DCt), hyperplasia (Hp), epithelium shortening (ES), curvature (Cu), fusion of lamellae (F), increased mucous secretion (Ms), and necrosis (N).

Table 2. Summarized histopathological effects of 20 µg/l CuO-NPs and Cu^{++} on the gill of guppy

Damages / Groups	An	DCt	Hp	N	ES	Cu	F	Ms
Exposure								
Control	+	+	+	-	-	+	-	-
CuO-NPs	+++	+	+++	++	++	-	++	++
Cu^{++}	++	++	+	+	+	++	+	+

None (−), mild (+), moderate (++) and severe (+++)
An: Aneurism; DCt: Dilated and clubbed tips; Hp: Hyperplasia; ES: Epithelium shortening; Cu: Curvature; F: Fusion of lamellae; Ms: Mucous secretion; N: Necrosis

The uptake potential of nanoparticles by aquatic organisms is one of the most important factors in assessing the toxicity of nanoparticles. The uptake of nanomaterials by the body of aquatic organisms depends on several parameters such as NPs size and shape, species, organs, route of exposure, environmental conditions, exposure duration, and exposure concentration.[19,23] We found a consistent trend towards Cu accumulation in gill tissue over time as well as higher Cu^{++} uptake compared to CuO-NPs. Bioaccumulation of nanoparticles such as CuO-NPs, ZnO-NPs, and TiO_2-NPs by fish and other aquatic species has been reported previously.[19,23,24] Accumulation of pollutants in gill tissue occurs as the result of competing rates between chemical accumulate and depurate. Hence, bioaccumulation of CuO-NPs and Cu^{++} can occur when the rate of accumulate is higher than the rate of depurate. Bioaccumulation of metal oxide NPs and other pollutants in gill tissue suggests that fish can be used as an appropriate indicator to assess pollutants in aquatic environment.[24-26] Gills have a large surface area, and thus, can greatly accumulate CuO-NPs and Cu^{++}.

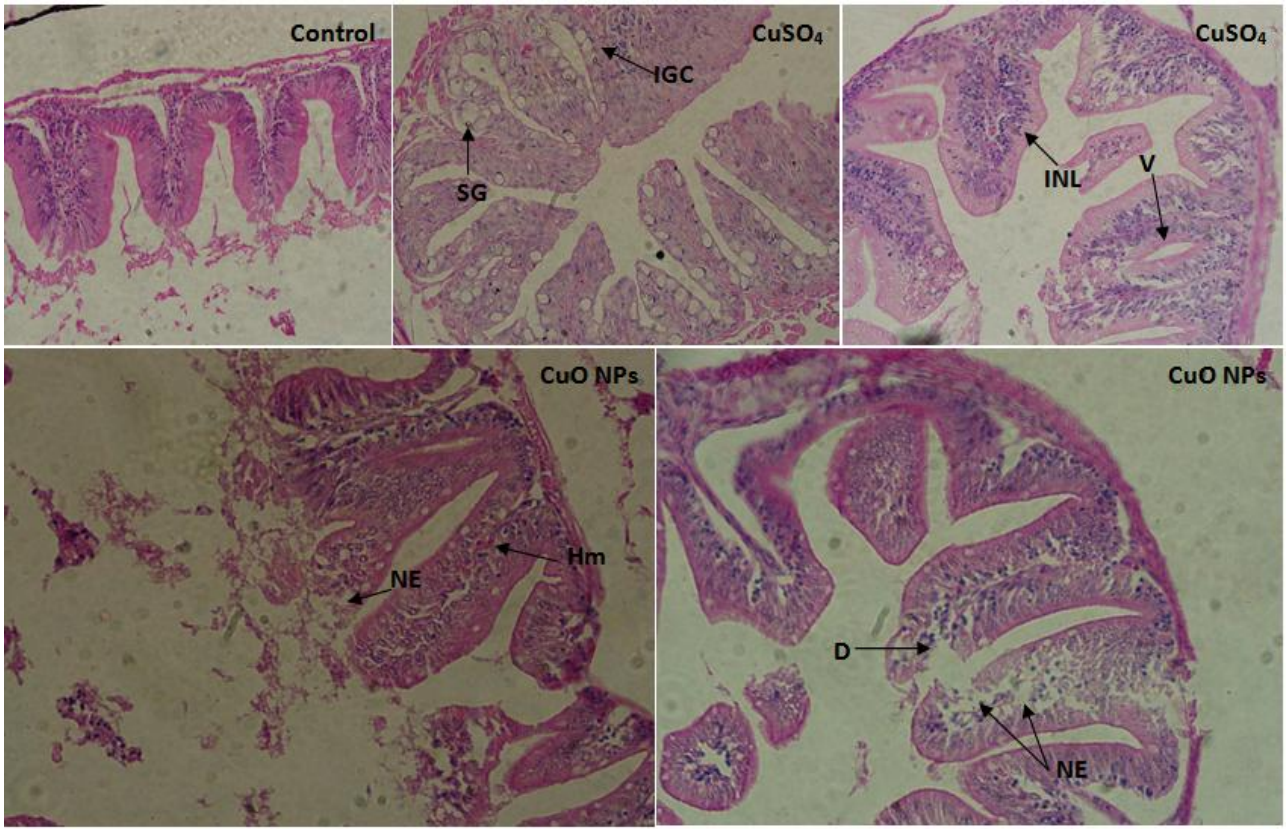

Figure 3. Histological alterations in the intestine of guppy after 10 days of exposure to CuO-NPs and Cu^{++} (x40)

The intestine of fish in the control group indicated only some minor histopathological alterations, whereas treatment groups showed injuries including degeneration (D), vacuolation (V), necrosis and erosion (NE), increase in the number of goblet cells (IGC), swelling of goblet cells (SG), hemorrhage (Hm), and increase in the number of lymphocyte (INL).

Table 3. Summarized histopathological effects of 20 µg/l CuO-NPs and Cu^{++} on the intestine of guppy

Damages / Groups	D	V	NE	IGC	SG	Hm	INL
Exposure							
Control	-	+	-	-	-	+	-
CuO-NPs	++	+	+++	+	+	+	+
Cu^{++}	+	++	+	++	+++	+	++

None (−); mild (+); moderate (++); severe (+++)

D: Degeneration; V: Vacuolation; NE: Necrosis and erosion; IGC: Increase in the number of goblet cells; SG: Swelling of goblet cells; Hm: Hemorrhage; INL: Increase in the number of lymphocyte

Histopathological changes are used to detect the effects of toxic substances on the organs of organisms.[27] Histopathological alteration in the gill may lead to the impairment of several functions, including respiration, osmoregulation, acid-base balance, and excretion of metabolite. Thus, gill histopathology appears to be a good biomarker for evaluating the effects of environmental stress on fish.[28,29] In the present study, aneurism, mucus, hyperplasia, and fusion of the filament lamellae were observed in the gills of guppy in both $CuSO_4$ and CuO-NPs experimental groups. Similar histopathological alterations have been reported as a result of exposure to copper sulphate[30,31] and copper nanoparticles[18] in other organisms. Al-Bairuty reported that exposure to copper nanoparticles resulted in edema, lamellar fusion, clubbed tips, and hyperplasia, aneurisms, and necrosis in the secondary lamellae of the gill filaments of

rainbow trout.[18] In the present study, significant differences were observed in gill histology of guppy between nanoparticulate and soluble forms of copper. The severity of histopathological lesions in the gill of guppy, such as aneurism, hyperplasia, fusion of lamellae, mucous secretion, and necrosis, was higher in the CuO-NPs treatment group than Cu^{++} treatment group. Moreover, exposure to CuO-NPs resulted in significantly greater thickening of the gill filaments. Griffitt et al. suggested that the effects of Cu-NPs and gill morphology are due to a combination of dissolution and particulate effect.[32] Cu-NPs exposure causes similar gill copper burdens as soluble copper exposure, this suggests that Cu-NPs is acting outer to the gill.

This study showed that CuO-NPs can cause lamellar fusion in gills. Lamellar fusion is a defence mechanism of fish gill that reduces total respiratory area when it is in contact with an external environment. This alteration could cause a decrease in oxygen-uptake for total metabolic activities, hence affecting the general health of fish.[27,33] Blanchard and Grosell[34] showed that exposure to copper can cause iono-regulatory impairment in fish due to its inhibitory effect on Na^+/K^+-ATPase activity causing proliferation of interlamellar cells in fish.[17] Moreover, studies suggest that the increased mucous secretion and hyperplasia of lamellae can make a barrier for NPs accumulated by gills and increase the diffusion distance for gas exchange.[5,35] In this study, we found aneurism to be a common alteration in gill resulted from exposure to both Cu^{++} and CuO-NPs. This is because of collapsing pillar cells in the secondary lamellae and swelling blood vessels and disturbances in blood flow in the gills.[33] It can therefore be concluded that necrosis of gill in guppy is the direct deleterious effect induced by CuO-NPs. This indicates that, for these forms of alteration, CuO-NPs are considerably more toxic than their sulphate equivalents.

The intestinal tissue is another main absorption site for chemical toxicants, such as nanoparticles, in fish. The intestine has the ability to uptake toxicants from ambient water[36] and may contribute to distribution of NPs in fish. This study showed histopathology lesions in intestinal organs including vacuolation, increase in the number of goblet cells, and swelling of goblet cells in the Cu^{++} treatment group, and degeneration, necrosis, and erosion in the CuO-NPs group. Perera and Pathiratne reported that Nile tilapia in the presence of TiO_2-NPs developed intestinal pathologies such as erosion of the villi epithelium, decline in mucous cells, and degeneration of the intestinal mucosa.[27] Federici et al. demonstrated that 14 days of exposure of rainbow trout to TiO_2-NPs caused several histopathological lesions in the intestine such as erosion of the villi, and fusion and vacuolation of the intestinal mucosa.[37] Both gill–blood route and intestine–blood route might contribute to the distribution of NPs in fish.[38] Nevertheless, to obtain a good knowledge of the distribution of NPs in organs of aquatic organisms and its mechanism in animals requires further studies.

Conclusion

In the present study, the effects of CuO-NPs and Cu^{++} were assessed on copper bioaccumulation in gill as well as histopathology of gill and intestine of guppy. The findings indicated that the Cu accumulation in the gill tissue was higher in the Cu^{++} exposure group than the CuO-NPs treatment group. However, the difference in the amount of copper uptake by the gill tissue between guppy fish in the Cu^{++} and CuO-NPs groups was not statistically significant. However, the severity of gill and intestine damages in the CuO-NPs exposed fish was higher than the Cu^{++} exposed fish. Therefore, it is recommended to be considered in toxicological study assessments in an aquatic environment.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

This work was supported by the Kurdistan University of Medical Sciences under Grant number 14/33858. The contribution of the Student Research Committee of Kurdistan University of Medical Sciences is sincerely appreciated.

References

1. Isani G, Falcioni ML, Barucca G, Sekar D, Andreani G, Carpene E, et al. Comparative toxicity of CuO nanoparticles and CuSO4 in rainbow trout. Ecotoxicol Environ Saf 2013; 97: 40-6.
2. Lapointe D, Pierron F, Couture P. Individual and combined effects of heat stress and aqueous or dietary copper exposure in fathead minnows (Pimephales promelas). Aquat Toxicol 2011; 104(1-2): 80-5.
3. Handy RD. Chronic effects of copper exposure versus endocrine toxicity: two sides of the same toxicological process? Comp Biochem Physiol A Mol Integr Physiol 2003; 135(1): 25-38.
4. Mela M, Guiloski IC, Doria HB, Rabitto IS, da Silva CA, Maraschi AC, et al. Risks of waterborne copper exposure to a cultivated freshwater Neotropical catfish (Rhamdia quelen). Ecotoxicol Environ Saf 2013; 88: 108-16.
5. Wang T, Long X, Liu Z, Cheng Y, Yan S. Effect of copper nanoparticles and copper sulphate on oxidation stress, cell apoptosis and immune responses in the intestines of juvenile Epinephelus coioides. Fish Shellfish Immunol 2015; 44(2): 674-82.
6. Scott GR, Sloman KA. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat Toxicol 2004; 68(4): 369-92.
7. Wang T, Long X, Cheng Y, Liu Z, Yan S. A comparison effect of copper nanoparticles versus copper sulphate on juvenile epinephelus coioides: growth parameters, digestive enzymes, body composition, and histology as biomarkers. Int J Genomics 2015; 2015: 783021.
8. Li Y, Liang J, Tao Z, Chen J. CuO particles and plates: Synthesis and gas-sensor application. Materials Research Bulletin 2004; 43(8-9): 2380-5.
9. Carnes CL, Klabunde KJ. The catalytic methanol synthesis over nanoparticle metal oxide catalysts. Journal of Molecular Catalysis A: Chemical 2003; 194(1-2): 227-36.
10. Sau TK, Rogach AL, Jackel F, Klar TA, Feldmann J. Properties and applications of colloidal nonspherical noble metal nanoparticles. Adv Mater 2010; 22(16): 1805-25.
11. Borkow G, Zatcoff RC, Gabbay J. Reducing the risk of skin pathologies in diabetics by using copper impregnated socks. Med Hypotheses 2009; 73(6): 883-6.
12. Borkow G, Gabbay J, Dardik R, Eidelman AI, Lavie Y, Grunfeld Y, et al. Molecular mechanisms of enhanced wound healing by copper oxide-impregnated dressings. Wound Repair Regen 2010; 18(2): 266-75.
13. Abdel-Khalek AA, Badran SR, Marie MS. Toxicity evaluation of copper oxide bulk and nanoparticles in Nile tilapia, Oreochromis niloticus, using hematological, bioaccumulation and histological biomarkers. Fish Physiol Biochem 2016.
14. Shaw BJ, Al-Bairuty G, Handy RD. Effects of waterborne copper nanoparticles and copper sulphate on rainbow trout, (Oncorhynchus mykiss): physiology and accumulation. Aquat Toxicol 2012; 116-117: 90-101.
15. Lakra WS, NagpureNS. Genotoxicological studies in fishes: A review. Indian J Anim Sci 2009; 79: 93-8.
16. Tao S, Liu C, Dawson R, Long A, Xu F. Uptake of cadmium adsorbed on particulates by gills of goldfish (Carassius auratus). Ecotoxicol Environ Saf 2000; 47(3): 306-13.
17. Griffitt RJ, Weil R, Hyndman KA, Denslow ND, Powers K, Taylor D, et al. Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (Danio rerio). Environ Sci Technol 2007; 41(23): 8178-86.
18. Al-bairuty GA. Histopathological effects of metal and metallic nanoparticles on the body systems of rainbow trout (Oncorhynchus mykiss) [PhD Thesis]. Plymouth, UK: School of Biomedical and Biological Sciences, University of Plymouth; 2013. p. 319.
19. Hao L, Chen L, Hao J, Zhong N. Bioaccumulation and sub-acute toxicity of zinc oxide nanoparticles in juvenile carp (Cyprinus carpio): a comparative study with its bulk counterparts. Ecotoxicol Environ Saf 2013; 91: 52-60.
20. Norouzi M, Mansouri B, Hamidian AH, Zarei I, Mansouri A. Metal concentrations in tissues of two fish species from Qeshm Island, Iran. Bull Environ Contam Toxicol 2012; 89(5): 1004-8.
21. Majnoni F, Rezaei M, Mansouri B, Hamidian AH. Metal concentrations in tissues of common carp, Cyprinus carpio, and silver carp, Hypophthalmichthys molitrix from the Zarivar Wetland in Western Iran. Archives of Polish Fisheries 2013; 21(1): 11-8.
22. Mansouri B, Maleki A, Johari SA, Reshahmanish N. Effects of cobalt oxide nanoparticles and cobalt ions on gill histopathology of zebrafish (Danio rerio). Aquaculture, Aquarium, Conservation & Legislation - International Journal of the Bioflux

Society 2015; 8(3): 438-44.
23. Tavana M, Kalbassi M R, Abedian Kenari A, Johari S A. Assessment of assimilation and elimination of silver and Tio_2 nanoparticles in artemia franciscana in different salinities. Journal of Oceanography 2014; 5(19): 91-103.
24. Zhao J, Wang Z, Liu X, Xie X, Zhang K, Xing B. Distribution of CuO nanoparticles in juvenile carp (Cyprinus carpio) and their potential toxicity. J Hazard Mater 2011; 197: 304-10.
25. Griffitt RJ, Luo J, Gao J, Bonzongo JC, Barber DS. Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ Toxicol Chem 2008; 27(9): 1972-8.
26. Mansouri B, Johari SA. Effects of short-term exposure to sublethal concentrations of silver nanoparticles on histopathology and electron microscope ultrastructure of zebrafish (danio rerio) gills. Iranian Journal of Toxicology 2016; 10(1): 15-20. [In Persian].
27. Perera S, Pathiratne A. Haemato-immunological and histological responses in Nile tilapia, Oreochromisniloticus exposed to titanium dioxide nanoparticles. Sri Lanka J Aquat Sci 2012; 17: 1-18.
28. Hassaninezhad L, Safahieh A, Salamat N, Savari A, Majd NE. Assessment of gill pathological responses in the tropical fish yellowfin seabream of Persian Gulf under mercury exposure. Toxicology Reports 2014; 1: 621-8.
29. da Cruz AL, Prado TM, Maciel LA, Couto RD. Environmental effects on the gills and blood of Oreochromis niloticus exposed to rivers of Bahia, Brazil. Ecotoxicol Environ Saf 2015; 111: 23-31.
30. Figueiredo-Fernandes A, Ferreira-Cardoso JV, Garcia-Santos S, Monteiro SM, Carrola J, Matos P, et al. Histopathological changes in liver and gill epithelium of Nile tilapia, Oreochromis niloticus, exposed to waterborne copper. Pesq Vet Bras 2007; 27(3): 103-9.
31. iraungkoorskul W, Sahaphong S, Kangwanrangsan N. Toxicity of copper in butterfish (Poronotus triacanthus): tissues accumulation and ultrastructural changes. Environ Toxicol 2007; 22(1): 92-100.
32. Griffitt RJ, Hyndman K, Denslow ND, Barber DS. Comparison of molecular and histological changes in zebrafish gills exposed to metallic nanoparticles. Toxicol Sci 2009; 107(2): 404-15.
33. Subashkumar S, Selvanayagam M. First report on: Acute toxicity and gill histopathology of fresh water fish Cyprinus carpio exposed to Zinc oxide (ZnO) nanoparticles. Int J Sci Res 2014; 4(3): 1-4.
34. Blanchard J, Grosell M. Copper toxicity across salinities from freshwater to seawater in the euryhaline fish Fundulus heteroclitus: is copper an ionoregulatory toxicant in high salinities? Aquat Toxicol 2006; 80(2): 131-9.
35. Scown TM, Santos EM, Johnston BD, Gaiser B, Baalousha M, Mitov S, et al. Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout. Toxicol Sci 2010; 115(2): 521-34.
36. Srinonate A, Banlunara W, Maneewattanapinyo P, Thammacharoen C, Ekgasit S, Kaewamatawong T. Acute toxicity study of nanosilver particles in tilapia (oreochromis niloticus): pathological changes, particle bioaccumulation and metallothionien protein expression. Thai J Vet Med 2015; 45(1): 81-9.
37. Federici G, Shaw BJ, Handy RD. Toxicity of titanium dioxide nanoparticles to rainbow trout (Oncorhynchus mykiss): gill injury, oxidative stress, and other physiological effects. Aquat Toxicol 2007; 84(4): 415-30.
38. Chen J, Dong X, Xin Y, Zhao M. Effects of titanium dioxide nano-particles on growth and some histological parameters of zebrafish (Danio rerio) after a long-term exposure. Aquat Toxicol 2011; 101(3-4): 493-9.

JAEHR Journal of Advances in Environmental Health Research

Simultaneous nitrification-denitrification in a sequencing batch reactor equipped with fixed Kaldnes carriers

Reza Darvishi Cheshmeh Soltani[1], Mahdi Safari[2], Reza Rezaee[2], Abbas Rezaee[3]

1 Department of Environmental Health, School of Health, Arak University of Medical Sciences, Arak, Iran
2 Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
3 Department of Environmental Health, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Original Article

Abstract

The main aim of this study was to evaluate the performance of a modified sequencing batch reactor (MSBR) using fixed Kaldnes carriers fed with acclimated sludge for ammonium removal via simultaneous nitrification-denitrification (SND) in synthetic wastewater. The results exhibited a SND of 82.3% within a 450-minute cycle time which was higher than that of a SBR without carrier (69.83%). Nitrite accumulation rate (NAR) increased from 16.94% to 32.83% until 120 minutes of cycle time, and then, decreased to 1.17% by 450 minutes. The biomass concentration in the bio-film (674 ± 6 mg/l) was lower than suspended biomass (1984 ± 12 mg/l). However, the specific oxygen uptake rate (SOUR) of the bio-film (5.24 ± 0.28 mg O_2/mg MLVSS.d) was greater than suspended biomass (1.89 ± 0.12 mg O_2/mg VSS.d), indicating the higher bioactivity of the bio-film than that of suspended biomass. Up to 3% salinity had no significant effect on MSBR performance for both chemical oxygen demand (COD) and ammonium removal. These results illustrated the high efficiency of the MSBR in the treatment of wastewater containing high salinity as well as the removal of nitrogen compounds via SND.

KEYWORDS: Nitrification, Denitrification, Salinity, Wastewater, Biomass

Date of submission: 12 May 2015, ***Date of acceptance:*** 22 Jul 2015

Citation: Darvishi Cheshmeh Soltani R, Safari M, Rezaee R, Rezaee A. **Simultaneous nitrification-denitrification in a sequencing batch reactor equipped with fixed Kaldnes carriers.** J Adv Environ Health Res 2015; 3(4): 224-34.

Introduction

In recent years, nitrate contamination of water resources due to discharge of domestic and industrial wastewater and extensive use of nitrogenous fertilizers has become a serious environmental concern.[1] The increasing concentration of nitrate in aquatic ecosystems can cause eutrophication (algal bloom).[2] In addition, high levels of nitrate in drinking water cause serious health problems such as methemoglobinemia in infants and gastric cancer.[3] Several methods, such as the reverse osmosis,[4] ion exchange,[5] electrochemical and bio-electrochemical processes,[1,6] adsorption,[7] electrocatalytic reduction,[8] and biological processes,[9] have been used for the removal of nitrate from aquatic environment. Biological nitrification-denitrification is the most commonly used process for nitrogen removal from wastewater.[10] However, it is more economical to combine nitrification and denitrification via a simultaneous nitrification-denitrification (SND) process in the same reactor than it is to perform the two processes separately.[11-14] Recently, SND process has been described for various wastewater treatment systems. These processes are used because they do not require an anoxic tank and they allow the pH to be maintained at a neutral level without the need for pH adjustment with an external acid-base

Corresponding Author:
Mahdi Safari
Email: safari.m.eng@gmail.com

0source.[14,15] Sequencing batch reactor (SBR) has been successfully used in wastewater treatment for chemical oxygen demand (COD) and nitrogen removal as an alternative to a conventional activated sludge system due to its advantages, including flexibility in operation, simplicity in structure, and the ability to meet many different treatment objectives.[16,17] This reactor has been successfully applied for SND process and even biological phosporous removal (BPR).[18-20] However, SBRs have a number of problems, such as low ability to settle sludge, high excess sludge production under high organic loading rates, and low removal efficiency due to their limitation in biomass production.[2,16,21,22] To solve these problems, different carriers have been used in the SBR in fixed or fluidized forms to increase the biomass concentration, and to overcome difficulties with respect to the maintenance of microorganisms and the slow growth of microorganisms. The support material is an important factor that keeps the microorganisms in the reactor by means of bio-film growth.[23] Over the past few years, researchers have studied municipal wastewater treatment using a bio-film SBR with different carriers.[22-25]

As a new alternative, in the present study, the Kaldnes carrier was applied for SND in the SBR. However, the application of this carrier for some biological systems such as SBR is limited due to its buoyancy. Hence, the Kaldnes carrier was packed, and then, placed in the SBR as a modified SBR (MSBR) to increase the micro-anoxic and micro-aerobic zone in the reactor for the enhancement of SND performance. To the best of our knowledge and based on our literature review, there is no report on the application of Kaldnes carrier as fixed-bed in the SBR for SND, together with the removal of COD in high saline wastewater treatment.

Materials and Methods

The experimental reactor was a rectangular tank made of Plexiglas with a working volume of 10 l and a free board of 5 cm. A flow diagram of the experimental reactor is shown in figure 1.

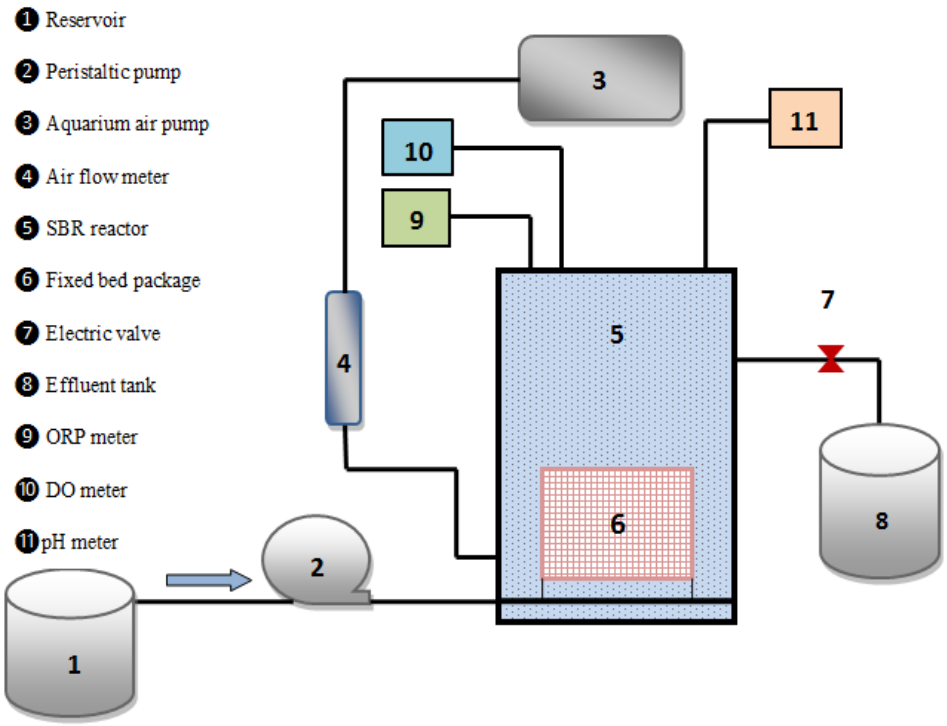

Figure 1. Schematic flow diagram of the experimental reactor

The reactor was provided with a peristaltic pump (Etatron, Italy), an air pump, an air flow meter (TG, Italy), and an electric valve. The MSBR reactor was filled with Kaldnes carriers (30% of the total volume of the MSBR) for growth of the bio-film. The length, diameter, density, and surface area of each carrier were 7 mm, 10 mm, 170 kg/m^3, and 24 m^2, respectively. The MSBR operation includes the four phases of filling (120 minutes), aeration (300 minutes), settling (30 minutes), and decanting (30 minutes), with a total time of 8 hours and 3 cycles in each day. The peristaltic pump was set to the flow rate of 1.5 l/hour to feed the wastewater into the reactor within a filling time of 120 minutes. The total volume of 3 l was exchanged in each cycle. Hydraulic retention time (HRT) was defined as the working volume of the SBR reactor divided by the total volume of synthetic wastewater fed into the reactor during one operational day.[2] Accordingly, a fixed HRT of 1600 minutes was attained on the basis of the total volume of 9 l fed into the reactor during one operational day and the reactor working volume of 10 l. These calculations are in accordance with the report of Wang et al.[20] The reactor was equipped with a portable dissolved oxygen (DO) meter (Jenway 9200, UK) and a portable oxidation-reduction potential (ORP) meter (ORP Tester 10, Malaysia). The timing of each phase (filling, aeration, settling, and decanting) of the reactor was controlled with adjustable timers. Moreover, using a portable pH meter (pH Tester 10, Malaysia), the pH of the system was maintained between 7.0 and 8.0.

The reactor was inoculated with activated sludge taken from return activated sludge line of a municipal wastewater treatment plant in Tehran, Iran. The reactor start-up involved daily feeding of synthetic wastewater until a biofilm started to form on the surface of carriers after 8 weeks. During this period, the reactor operated in a "fill and draw" mode for the formation of a bio-film layer on the Kaldnes surface. The COD:N:P ratio in the wastewater during the acclimation period was kept at 100:5:1. In the "fill and draw" mode, ammonium, COD, mixed liquid suspended solid (MLSS), and sludge volume index (SVI) were measured daily. After measurement, the air pump was switched off and the sludge was allowed to settle at the bottom of the reactor. Then, 10% of the volume of the reactor was withdrawn and replaced with synthetic wastewater consisting of $MgSO_4.7H_2O$ (69.6 mg/l), $FeCl_2 \cdot 4H_2O$ (17.25 mg/l), $CaCl_2 \cdot 2H_2O$ (22.5 mg/l), $CuSO_4 \cdot H_2O$ (0.08 mg/l), $Na_2MoO_4 \cdot 2H_2O$ (0.15 mg/l), $MnSO_4 \cdot H_2O$ (0.13 mg/l), $ZnCl_2$ (0.23 mg/l), and $CoCl_2 \cdot 6H_2O$ (0.42 mg/l).[26] The ammonium and COD concentrations reached 25 mg NH_4-N/l and 500 mg/l, respectively, and aeration was resumed. After the acclimation period, the MLSS and SVI reached approximately 2000 mg/l and 125 ml/g, respectively. Once the system reached steady state conditions and a suitable bio-film had grown on the carriers, the reactors were operated in series in a cyclic test mode. Ethanol (C_2H_5OH), ammonium chloride (NH_4Cl), and phosphate hydrogen potassium (HK_2PO_4) were used as carbon, nitrogen, and phosphorus sources, respectively. The C:N:Pratio in the simulated wastewater was similar to that in the "fill and draw" mode.

COD, MLSS, volatile MLSS (MLVSS), and SVI were determined according to standard methods for the examination of water and wastewater.[27] SVI was calculated as ml/g by dividing the results of the settling test (ml) by the MLSS concentration in the reactor. Samples were filtered with 0.45 µm filters, and then, NH_4-N, NO_3-N, and NO_2-N contents were colorimetrically measured using standard methods with a UV-Vis spectrophotometer (Unico 2100) at 425, 220, and 543 nm, respectively. In the case of NO_3-N measurement, the interference of the organic matter was eliminated using the following equation:

$$A_{corr} = A_{220} - 2 \times A_{275} \quad (1)$$

where A_{corr}, A_{220}, and A_{275} are the corrected UV-light absorbance of nitrate,

absorbance at 220 nm, and absorbance at 275 nm, respectively.[28] Collected samples were acidified by adding 0.2 ml H_2SO_4 in a concentrated form to 100 ml of each sample and stored at 4 °C for analysis on the next day.[29] A scanning electron microscope (SEM) (Philips XL 30, the Netherlands) was applied to study the morphological details on the bio-film surface. DO, pH, and ORP were measured using specific probes.

To estimate the amount of bio-film attached to the surface of each carrier, 20 carriers were selected randomly from the reactor. The carriers were separated from the reactor and dried until constant weight was achieved. The dried carriers were weighed to calculate the total mass (M*t*) of the carriers with bio-film. Then, the net weight of the carriers without bio-film (M*c*) was estimated after washing and cleaning the carriers. After that, the amount of bio-film attached to the surface of 20 carriers (BS_{20}) was calculated according to equation 2.

$$BS_{20} = M_t - M_c \quad (2)$$

According to the total number of carriers in the package of the reactor (861), the total amount of bio-film in the reactor (BS) can be estimated using equation 3.[25]

$$BS = BS_{20} \times \frac{861 L^{-1}}{20} \quad (3)$$

The specific oxygen uptake rate (SOUR) was used to evaluate the bioactivity of the bio-film attached to the surface of the Kaldnes carriers. The SOUR test was performed in Erlenmeyer flasks equipped with DO meters. Randomly, 10 carriers and 100 ml of mixed liquid suspended solid were removed from the reactor and stored in two separate flasks for comparison. First, the Erlenmeyer flasks were aerated until an initial concentration of about 8 mg O_2/l was achieved. Then, the reduction in the oxygen content was monitored and recorded via DO meter. The analysis was terminated when the DO concentration decreased to about 1 mg/l. Ethanol was used as the carbon source and was added at a 200 mg/l COD concentration to each flask. Finally, the SOUR was calculated by dividing the oxygen uptake rate (OUR) by the MLVSS concentration.[30,31]

Results and Discussion

Acclimation period and bio-film growth

Biomass acclimation has been a key factor for improving nitrification performance in biological reactors.[32] In this study, acclimation was carried out for 30 days with 500 mg/l COD and 25 mg NH_4-N/l. The variations in SVI and MLSS versus the effluent COD and NH_4-N during the 30 days of the acclimation period are shown in figure 2. During this period, effluent COD and NH_4-N concentrations reached 5.57 and 1.05 mg/l, respectively. As can be seen in figure 2, the decrease in COD and NH_4-N concentrations was achieved through the reduction of MLSS concentration from 6500 to 1950 mg/l, while the SVI increased from 51 to 123 ml/g.

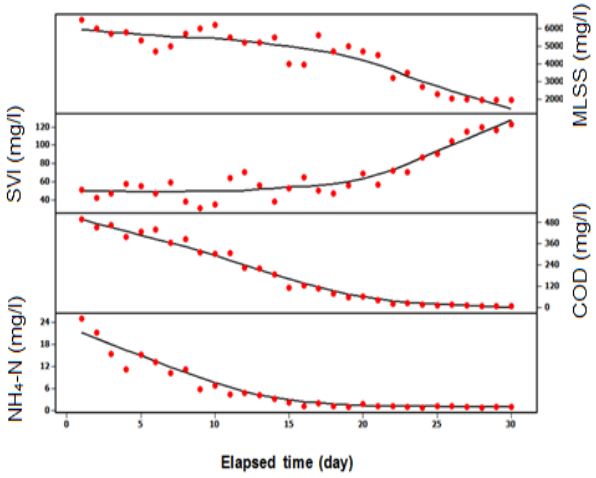

Figure 2. Variations in chemical oxygen demand (COD) and ammonium versus variations in sludge volume index (SVI) and mixed liquid suspended solid (MLSS) during acclimation period (Initial COD concentration = 500 mg/l, initial ammonium concentration = 25 mg NH_4-N/l)

Surface Morphology of the attached bio-film onto the carrier

Figure 3 [(a-1) and (a-2)] shows the structure of the upper and lower surface of the Kaldnes

carrier without bio-film at 5000X magnification and displays a smooth carrier surface for bio-film growth. The SEM image in figure 3 shows the fully grown bio-film on the surface of the carriers. Moreover, according to figure 3 (b-1), the presence of bacteria as attached bio-film is evident, thus indicating that an appropriate environment for the removal of pollutants by microorganisms has been achieved. This structure can also create many suitable anoxic and aerobic micro-zones to enhance SND processes in the same reactor. According to figure 3 (b-2), after the experiments related to salinity, the bio-film structure was not considerably different from the structure shown in figure 3 (b-1), implying the resistance of bio-film against undesirable conditions such as salinity.

Bio-film growth

The concentration of biomass attached onto the surface of the Kaldnes carrier was calculated according to equations (3) and (4). Moreover, the amount of suspended biomass in the reactor was calculated according to the method described in standard methods for the examination of water and wastewater.[27] The biomass concentration of the bio-film and the suspended biomass in the reactor were 674 ± 5 and 1984 ± 12 mg/l, respectively. The biomass concentration of the bio-film attached onto the surface of the media was lower than that of suspended biomass. Nevertheless, the ammonium removal efficiency of the SBR with the carrier was higher than that of the SBR. This finding shows that the bio-film had higher bioactivity than the suspended biomass.

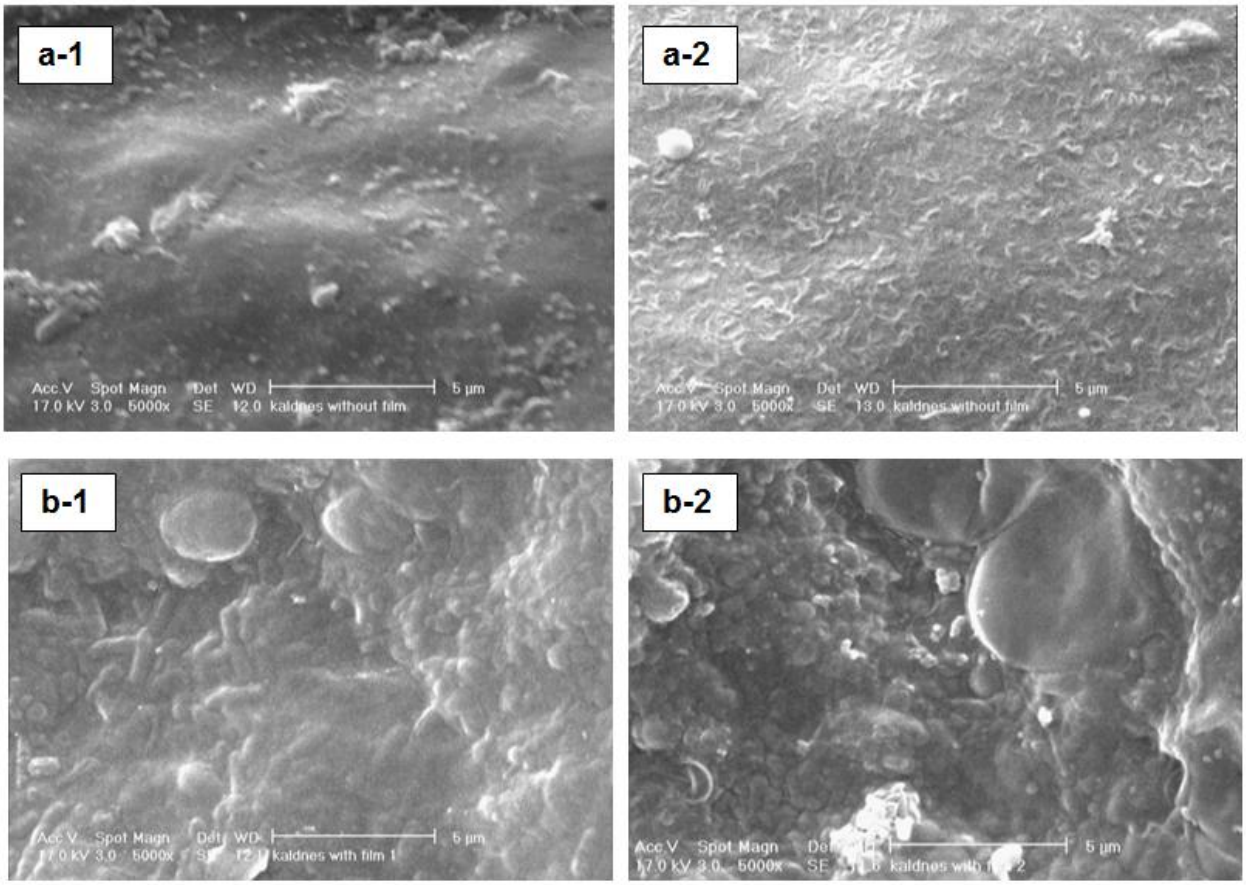

Figure 3. Scanning electron microscopy (SEM) images of the (a-1) upper and (a-2) lower surface of the carrier without bio-film, (b-1) the carrier with bio-film and (2-b) the surface morphology of the bio-film after ammonium and chemical oxygen demand (COD) removal in saline wastewater taken at 5000 X magnification

Specific oxygen uptake rate (SOUR)

The SOURs of the bio-film and the suspended biomass were 5.24 ± 0.28 and 1.89 ± 0.12 mg O_2/mg MLVSS.d, respectively. The SOUR for the attached and suspended biomass showed that the respirations and catabolic activities of the microorganisms in the bio-film were higher than those of the suspended biomass. This indicates the high bioactivity of the microorganisms attached onto the Kaldnes surfaces. Chen et al. showed that the granules in a SBR reactor have better bioactivity in terms of SOUR than the suspended sludge.[33]

Simultaneous nitrification-denitrification process in MSBR

To treat synthetic wastewater that contains ethanol as the carbon source, the evaluation of the removal of NH_4-N and NO_3-N were carried out using a SND process during an operational cycle in a MSBR reactor. Combining nitrification and denitrification for complete ammonium removal in the same tank is an efficient way to reduce the operational requirement related to separate processes in treating wastewater.[34] Variations of NH_4-N, NO_3-N, and NO_2-N in cycle time are depicted in figure 4. As shown in figure 4, increased NO_3-N concentration during the aeration phase and then decrease in the produced NO_3-N at the end of the settling phase confirms the transformation of NH_4-N to N_2 gas. Furthermore, as seen at the end of the settling phase, NH_4-N and NO_2-N concentrations reached the lowest values, indicating N_2 gas production as a result of SND. This fact was also demonstrated in the investigation by Rodriguez et al.[19] At the beginning of the settling phase, the concentration of nitrate decreased because of the absence of aeration at a lower oxidation reduction potential (ORP). ORP is commonly studied for controlling and monitoring SND process because of the electromotive force developed when oxidizers or reducers are present in wastewater.[35] Favorable denitrification usually occurs along with decreasing ORP. ORP increases during aeration at high DO concentration, and then, decreases during the anoxic phase.[36]

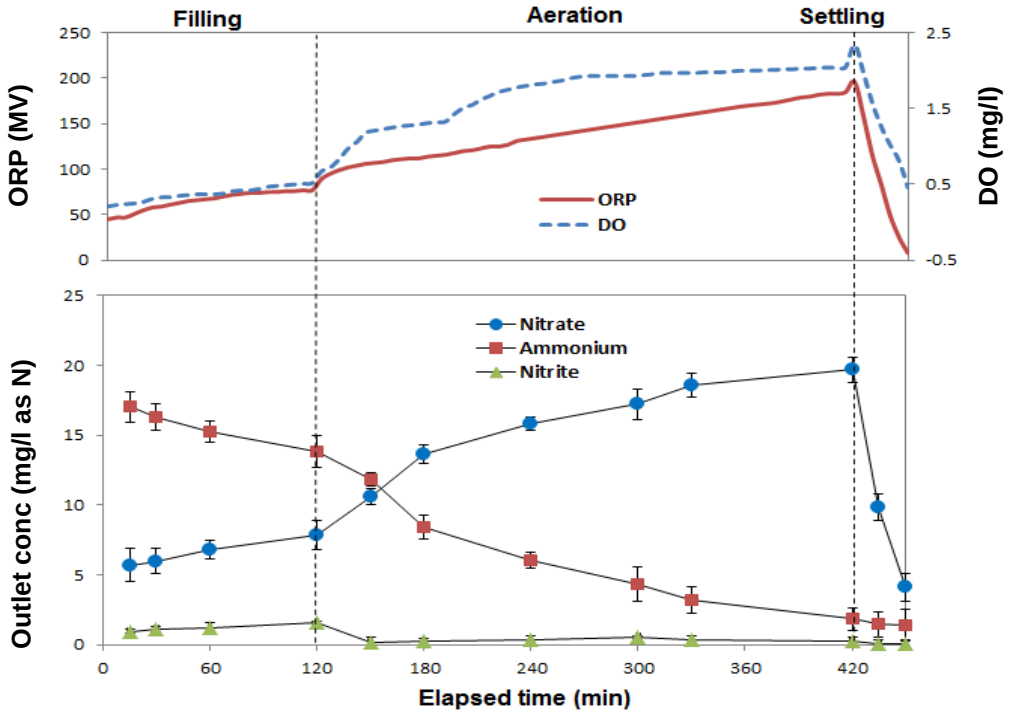

Figure 4. Variations in the nitrogen species concentration, and dissolved oxygen (DO) and oxidation-reduction potential (ORP) values versus elapsed time (Initial ammonium concentration = 25 mg NH_4-N/l)

Figure 4 shows the profile of DO and ORP during the SND to evaluate the effect of oxidative conditions in the reactor for simultaneous removal. As shown in figure 4, the greatest ORP decline zone occurred at the end of the settling phase when the dissolved oxygen reached its lowest value. At this point, the DO and ORP values were 0.46 mg/l and 8 mV, respectively. Moreover, figure 4 shows that the highest ORP that occurred at approximately the end of the aeration phase with a value of 196 mV matched the high DO concentration (2.34 mg/l). Thus, we can use ORP variations in the system as an efficient means for evaluation of oxidative and reductive conditions. Comparatively, a DO concentration between 2.5 and 4.0 mg/l was recommended for SND in a study conducted by Li et al.[11] They used an aerobic sequencing batch biofilm reactor (SBBR) packed with Bauer rings, which had higher energy-consumption than the present work. Subsequently, by using MSBR, they evaluated the feasibility of SND.[11] The following equation was used to calculate the efficiency of SND in the MSBR reactor.[18]

$$\text{Efficiency}_{\text{SND}}(\%) = \left(1 - \frac{\text{NO}^{-}_{\text{x remained}}}{\text{NH}^{+}_{4\,\text{oxidized}}}\right) \times 100 \qquad (4)$$

where $\text{NO}^{-}_{\text{x remained}}$ is the remaining NO_3-N/NO_2-N and $NH^{+}_{4\,oxidized}$ is the oxidized NH_4-N after the reaction. As seen in figure 4, by increasing reaction time to 450 minutes (at the end of the settling phase), the effluent concentrations of NH_4-N, NO_3-N, and NO_2-N reached 1.43, 4.12, and 0.05 mg/l N, respectively. It seems that using a fixed bed Kaldnes carrier is appropriate for SND in a MSBR reactor. In the SBR reactor equipped with the Kaldnes carriers, the biofilm becomes thicker, the SND efficiency improves, and the effluent concentration is maintained at a low level.[37] It has been demonstrated that reduction in SND efficiency in conventional SBR with suspended biomass can be attributed to lower anoxic microzones within the flocs which are maximized in the bio-film attached onto Kaldnes carriers.[38] This statement was in agreement with the findings of Li et al., who found that thicker bio-film is beneficial for SND.[11] We know that ticker bio-film results in increased anoxic microzones within the film. It has been found that high concentrations of nitrite have a negative effect on the removal of ammonium via SND.[13] Therefore, to evaluate the performance of the reactor for ammonium oxidation, the nitrite accumulation rate (NAR) was estimated using equation 5.[15]

$$\text{NAR}(\%) = \frac{\text{NO}_2\text{-N}}{\text{NO}_2\text{-N} + \text{NO}_3\text{-N}} \times 100 \qquad (5)$$

Accordingly, the variations of NAR and nitrite are depicted in figure 5. Figure 5 shows a low NAR% during SND. NAR increased from 16.94% to 32.83% (equal to increasing nitrite from 0.96 to 1.58 mg NO_2-N/l) as the reaction time increased from beginning to 120 minutes. Then, NAR decreased from 32.83% to 1.17% (equal to decreasing nitrite from 1.58 to 0.05 mg NO_2-N/l) as the time increased from 120 minutes to 450 minutes. It was observed in another investigation that ammonium can be converted to N_2 gas without the accumulation of nitrite by the mixed culture during SND.[34] It has been confirmed that nitrite can be completely converted in the presence of sufficient carbon source during the SND. Zhang et al., in their study, observed that nitrite conversion increased from 27.59% at C/N of 5 to 100% at C/N of 20.[13] Our operational conditions regarding C/N ratio were consisted with the report of Zhang et al.[13] Therefore, sufficient C/N ratio, which was applied in the present work, was beneficial in terms of low accumulation of nitrite in the reactor during the SND. At the end of the experiments, the package of carriers was removed from the reactor and the SND efficiency was measured. This enabled the evaluation of the effects of the Kaldnes carrier on SND efficiency. At the end of the settling phase, the effluent concentrations of NH_4-N, NO_3-N, and NO_2-N reached 4.65, 6.05 and 0.09 mg/l N,

respectively. According to equation 4, SND using SBR without Kaldnes carriers would be 69.83% which is lower than that of MSBR. This demonstrates that the capability of the MSBR for SND is higher than the SBR without the carrier.

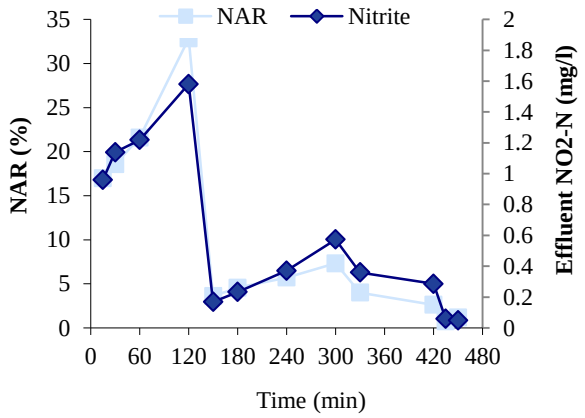

Figure 5. Variations in the effluent concentration of NO_2-N and nitrite accumulation rate (NAR) (%) versus reaction time

Effect of salinity on SND

High saline wastewater induces salt stress in the microbial communities in biological wastewater systems. Salinity can cause inhibition for many enzymes, inactivation of bacteria, and plasmolysis.[29] For example, metal refinery wastewater has high salinity which is difficult to treat biologically.[39] In this set of experiments, ammonium removal from saline wastewaters was investigated in MSBR over 62 days because of the negative effect of salinity on nitrification compared to denitrification. For example, in a study related to denitrification at high salinity, a high denitrification rate was achieved at 0%–10% NaCl.[39] The effects of salt concentration (0.5%–5% NaCl) on ammonium and COD removal efficiencies in MSBR were investigated. To evaluate the salinity effect, the MSBR cycle was fixed at 8 hours, as stated in the materials and methods section. To compare SBR with and without the media, ammonium and COD were adjusted to 50 mg NH_4-N/l and 1000 mg/l, respectively. As shown in figure 6, increasing the salinity from 0.5 to 5% led to the decrease of the removal percentage of ammonium and COD from 70.45% to 15.1% and from 99.05% to 28.12%, respectively. However, the COD removal percentage was higher than 90% for all tested salinity concentrations up to 1%.

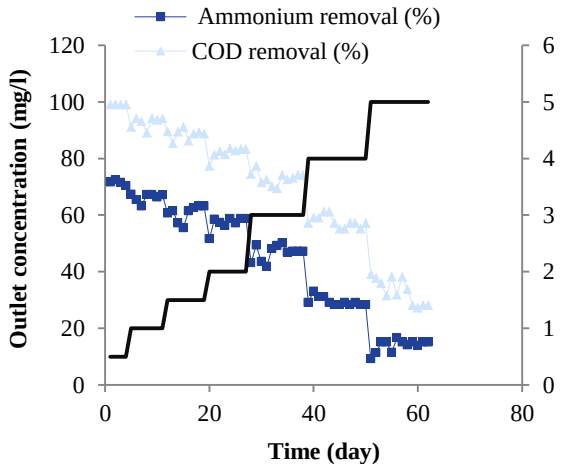

Figure 6. Effect of salinity on the removal percentage of ammonium and chemical oxygen demand (COD) during 62 days of operation (Initial COD concentration = 1000 mg/l, initial ammonium concentration = 50 mg NH_4-N/l)

With the increasing of salinity up to 3%, a significant decrease was observed in ammonium and COD removal percentages. At 4% salinity, COD and ammonium removal percentages reached 57.12 and 29.11%, respectively. Figure 6 shows the tolerance of the MSBR to salinity, up to 3%, for treating synthetic wastewater containing 50 mg NH_4-N/l and 1000 mg COD/l. The obtained results show that MSBR is suitable for treating wastewater containing high salinity. These results indicate that a sufficient acclimation period (62 days) and suitable growth of bio-film on the Kaldnes carriers can help overcome the stressing and inhibitory conditions related to high salinity in synthetic wastewater.[40] Biomass retention within a reactor by means of a carrier is a key factor in the prevention of washout of slow-growing nitrification bacteria, which can be more resistant to inhibitory conditions, such as salinity. Results obtained in this study were consistent with those attained by Bassin et al.[32] Their study showed a high

nitrification percentage for municipal wastewater with salinity concentrations of up to 8000 mg/l, equal to 0.8% by means of SBR containing a carrier. Rene et al. performed a study on treating fish market wastewater by SBR and showed that increasing the salt concentration to 3% lowered the nitrogen removal percentage by 35% to 45%.[29] This effect was attributed to salt induced forces. After the evaluation of the effect of salinity on ammonium and COD removal in synthetic wastewater in a MSBR reactor, a sample of carrier was withdrawn and analyzed through SEM to determine the effect of salinity on bio-film. As shown in figure 3 (b-2), after the experiments related to salinity, the bio-film structure was not considerably different from the structure shown in figure 3 (b-1). However, other researchers have reported that salinity may potentially disturb bio-film compositions, such as extracellular polymeric substances, and can affect the oxygen transfer rate.[29]

Conclusion

This investigation confirmed the suitability of Kaldnes as a polyethylene carrier in a MSBR for a SND process. A close relationship was observed between decreasing ORP and nitrate reduction during SND in MSBR. SND results indicated that the SND efficiency of the MSBR was higher than that of SBR without Kaldnes carriers. The biomass concentration of the bio-film attached onto the carrier was lower than that of suspended biomass, but the higher SOUR of the attached biomass compare to the suspended biomass concentration indicated a higher bioactivity than the suspended biomass. Salinity had a low effect on decreasing the removal percentage, which ranged between 1% to 3% salinity. Finally, it should be stated that the application of SBR with Kaldnes carriers can be an effective way to treat wastewater containing high salinity and COD levels as well as to remove nitrogen compounds via SND.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

The authors would like to thank Arak University of Medical Sciences, Iran, and Kurdistan University of Medical Sciences, Iran, for their support.

References

1. Safari M, Rezaee A, Ayati B, Jonidi-Jafari A. Simultaneous removal of nitrate and its intermediates by use of bipolar electrochemistry. Res Chem Intermed 2013; 41(3): 1395-72.
2. Darvishi Cheshmeh Soltani R, Rezaee A, Godini H, Khataee AR, Hasanbeiki A. Photoelectrochemical treatment of ammonium using seawater as a natural supporting electrolyte. Chem Ecol 2013; 29(1): 72-85.
3. Rezaee A, Safari M, Hossini H. Bioelectrochemical denitrification using carbon felt/multiwall carbon nanotube. Environ Technol 2015; 36(5-8): 1057-62.
4. Epsztein R, Nir O, Lahav O, Green M. Selective nitrate removal from groundwater using a hybrid nanofiltration-reverse osmosis filtration scheme. Chem Eng J 2015; 279: 372-8.
5. Alikhani M, Moghbeli MR. Ion-exchange polyHIPE type membrane for removing nitrate ions: Preparation, characterization, kinetics and adsorption studies. Chem Eng J 2014; 239: 93-104.
6. Safari M, Rezaee A, Ayati B, Jonidi-Jafari A. Bio-electrochemical reduction of nitrate utilizing MWCNT supported on carbon base electrodes: A comparison study. J Taiwan Inst Chem Eng 2014; 45(5): 2212-6.
7. Mukherjee R, De S. Adsorptive removal of nitrate from aqueous solution by polyacrylonitrile□alumina nanoparticle mixed matrix hollow-fiber membrane. J Memb Sci 2014; 466: 281-92.
8. Siriwatcharapiboon W, Kwon Y, Yang J, Chantry RL, Li Z, Horswell SL, et al. Promotion effects of Sn on the electrocatalytic reduction of nitrate at Rh nanoparticles. Chem Electro Chem 2014; 1(1): 172-9.
9. Chen Y, Wang D, Zhu X, Zheng X, Feng L. Long-term effects of copper nanoparticles on wastewater biological nutrient removal and N_2O generation in the activated sludge process. Environ Sci Technol 2012; 46(22): 12452-8.
10. Petrovic A, Simonic M. Effect of Chlorella sorokiniana on the biological denitrification of drinking water. Environ Sci Pollut Res Int 2015; 22(7): 5171-83.
11. Li J, Peng Y, Gu G, Wei S. Factors affecting simultaneous nitrification and denitrification in an SBBR treating domestic wastewater. Front Environ Sci En 2007; 1(2): 246-50.
12. Wu C, Chen Z, Liu X, Peng Y. Nitrification-denitrification via nitrite in SBR using real-time

control strategy when treating domestic wastewater. Biochem Eng J 2007; 36(2): 87-92.
13. Zhang Y, Shi Z, Chen M, Dong X, Zhou J. Evaluation of simultaneous nitrification and denitrification under controlled conditions by an aerobic denitrifier culture. Bioresour Technol 2014; 175C: 602-5.
14. Zhang L, Wei C, Zhang K, Zhang C, Fang Q, Li S. Effects of temperature on simultaneous nitrification and denitrification via nitrite in a sequencing batch biofilm reactor. Bioprocess Biosyst Eng 2009; 32(2): 175-82.
15. Wang J, Peng Y, Wang S, Gao Y. Nitrogen removal by simultaneous nitrification and denitrification via nitrite in a sequence hybrid biological reactor. Chin J Chem Eng 2008; 16(5): 778-84.
16. Du R, Peng Y, Cao S, Wu C, Weng D, Wang S, et al. Advanced nitrogen removal with simultaneous Anammox and denitrification in sequencing batch reactor. Bioresour Technol 2014; 162: 316-22.
17. Li XM, Chen HB, Yang Q, Wang DB, Luo K, Zeng GM. Biological nutrient removal in a sequencing batch reactor operated as oxic/anoxic/extended-idle regime. Chemosphere 2014; 105: 75-81.
18. Chiu YC, Lee LL, Chang CN, Chao AC. Control of carbon and ammonium ratio for simultaneous nitrification and denitrification in a sequencing batch bioreactor. Int Biodeterior Biodegradation 2007; 59(1): 1-7.
19. Rodriguez DC, Pino N, Penuela G. Monitoring the removal of nitrogen by applying a nitrification-denitrification process in a Sequencing Batch Reactor (SBR). Bioresour Technol 2011; 102(3): 2316-21.
20. Wang D, Zheng W, Li X, Yang Q, Liao D, Zeng G. Evaluation of the feasibility of alcohols serving as external carbon sources for biological phosphorus removal induced by the oxic/extended-idle regime. Biotechnol Bioeng 2013; 110(3): 827-37.
21. Darvishi Cheshmeh Soltani R, Rezaee RA, Godini H, Khataee AR, Jorfi S. Organic matter removal under high loads in a fixed-bed sequencing batch reactor with peach pit as carrier. Environ Prog Sustain Energy 2013; 32(3): 681-7.
22. Sirianuntapiboon S, Yommee S. Application of a new type of moving bio-film in aerobic sequencing batch reactor (aerobic-SBR). J Environ Manage 2006; 78(2): 149-56.
23. Garcia ML, Lapa KR, Foresti E, Zaiat M. Effects of bed materials on the performance of an anaerobic sequencing batch biofilm reactor treating domestic sewage. J Environ Manage 2008; 88(4): 1471-7.
24. Zhan XM, Rodgers M, O'Reilly E. Biofilm growth and characteristics in an alternating pumped sequencing batch biofilm reactor (APSBBR). Water Res 2006; 40(4): 817-25.
25. Jing JY, Feng J, Li WY, Xu Y. Removal of COD from coking-plant wastewater in the moving-bed biofilm sequencing batch reactor. Korean J Chem Eng 2009; 26(2): 564-8.
26. Zhang LL, Chen JM, Fang F. Biodegradation of methyl t-butyl ether by aerobic granules under a cosubstrate condition. Appl Microbiol Biotechnol 2008; 78(3): 543-50.
27. Eaton AD, Franson MA. Standard methods for the examination of water & wastewater. Washington, DC: American Public Health Association; 2005.
28. Parvanova-Mancheva T, Beschkov V. Microbial denitrification by immobilized bacteria Pseudomonas denitrificans stimulated by constant electric field. Biochem Eng J 2009; 44(2-3): 208-13.
29. Rene ER, Kim SJ, Park HS. Effect of COD/N ratio and salinity on the performance of sequencing batch reactors. Bioresour Technol 2008; 99(4): 839-46.
30. Obaja D, Mace S, Costa J, Sans C, Mata-Alvarez J. Nitrification, denitrification and biological phosphorus removal in piggery wastewater using a sequencing batch reactor. Bioresour Technol 2003; 87(1): 103-11.
31. Wang RC, Wen XH, Qian Y. Influence of carrier concentration on the performance and microbial characteristics of a suspended carrier biofilm reactor. Process Biochem 2005; 40(9): 2992-3001.
32. Bassin JP, Dezotti M, Sant'anna GL Jr. Nitrification of industrial and domestic saline wastewaters in moving bed biofilm reactor and sequencing batch reactor. J Hazard Mater 2011; 185(1): 242-8.
33. Chen Y, Jiang W, Liang DT, Tay JH. Biodegradation and kinetics of aerobic granules under high organic loading rates in sequencing batch reactor. Appl Microbiol Biotechnol 2008; 79(2): 301-8.
34. Du G, Geng J, Chen J, Lun S. Mixed culture of nitrifying bacteria and denitrifying bacteria for simultaneous nitrification and denitrification. World J Microbiol Biotechnol 2003; 19(4): 433-7.
35. Gao D, Peng Y, Li B, Liang H. Shortcut nitrification-denitrification by real-time control strategies. Bioresour Technol 2009; 100(7): 2298-300.
36. Li B, Irvin S. The comparison of alkalinity and ORP as indicators for nitrification and denitrification in a sequencing batch reactor (SBR). Biochem Eng J 2007; 34(3): 248-55.
37. Yang S, Yang F, Fu Z, Wang T, Lei R. Simultaneous nitrogen and phosphorus removal by a novel sequencing batch moving bed membrane bioreactor for wastewater treatment. J Hazard Mater 2010; 175(1-3): 551-7.
38. Holman JB, Wareham DG. COD, ammonia and dissolved oxygen time profiles in the simultaneous

nitrification/denitrification process. Biochem Eng J 2005; 22(2): 125-33.
39. Osaka T, Shirotani K, Yoshie S, Tsuneda S. Effects of carbon source on denitrification efficiency and microbial community structure in a saline wastewater treatment process. Water Res 2008; 42(14): 3709-18.
40. Dincer AR, Kargi F. Salt inhibition kinetics in nitrification of synthetic saline wastewater. Enzyme Microb Technol 2001; 28(7-8): 661-5.

Journal of Advances in Environmental Health Research

Estimation of target hazard quotients for heavy metals intake through the consumption of fish from Sirvan River in Kermanshah Province, Iran

Borhan Mansouri[1], Afshin Maleki[2], Behrouz Davari[3], Jamal Karimi[4], Vahid Momeneh[5]

1 Student Research Committee AND Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
2 Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
3 Department of Entomology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
4 Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
5 Kermanshah Regional Water Authority, Kermanshah, Iran

Original Article

Abstract

The aim of this research was to investigate concentrations of cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), and zinc (Zn) in the muscle, gill, and liver of common carp (Cyprinus carpio), tuwini (Capoeta trutta), and Grass carp (Ctenopharyngodon idella) from Sirvan River, Kermanshah Province, Iran, during November to December 2014. This investigation was conducted in order to determine the potential health risk of the intake of these metals through the consumption of the edible parts of fish and also to assess the safe dietary intake levels of these metals. The results of the present study indicated that the highest and lowest accumulated metal concentrations were related to Zn and Cd, respectively. Moreover, the metal concentrations in the gill and liver were higher than in the muscles of the three fish species. The target hazard quotients (THQs) for an adult with mean body weight of 71.5 kg were below 1 based on Cd, Pb, Cr, Cu, and Zn levels. In conclusion, the obtained results indicated that the levels of metals in the edible muscle of fish species in this study were below the level of concern for human consumption.

KEYWORDS: Gills, Liver, Metals, Carp, Body Weight

Date of submission: 22 May 2015, ***Date of acceptance:*** 19 Jul 2015

Citation: Mansouri B, Maleki A, Davari B, Karimi J, Momeneh V. **Estimation of target hazard quotients for heavy metals intake through the consumption of fish from Sirvan River in Kermanshah Province, Iran.** J Adv Environ Health Res 2015; 3(4): 235-41.

Introduction

Over the past decade, pollution of freshwater ecosystems by heavy metals has become a problem on local, regional, and global scales. Heavy metals are toxicants, and highly persistent and nonbiodegredable contaminants, and may be accumulated in the human food chain.[1,2] Moreover, heavy metals are divided into two important groups; essential metals and nonessential metals. Metals such as copper (Cu) and zinc (Zn) are essential elements for growth and development of the body, while other metals such cadmium (Cd), lead (Pb), and chromium (Cr) are nonessential elements for the body and have no known role in biological and physiological mechanisms.[3,4] Several studies have indicated that Cd and Pb are toxic heavy metals for humans and other organisms and affect a number of organs and systems such as the kidney, nerve tissue, circulatory system, reproductive system, and immune systems.[5,6] Thus, an important step in monitoring freshwater ecosystems is knowledge of the metal levels

Corresponding Author:
Afshin Maleki
Email: maleki47@yahoo.com

in the organs of aquatic organisms regarding both aquatic ecosystems management and human consumption of aquatic organisms.

Numerous researches have assessed metal levels in aquatic ecosystems using different types of aquatic organisms such as seaweeds and filter-feeding molluscs,[7,8] shrimp and crab,[9,10] mussels and oysters,[11,12] and fish.[1,13] Fish is a source of minerals, protein, and omega-3 polyunsaturated fatty acids for human consumption and has a positive impact on cardiovascular disease, inflammatory conditions, and brain and immune system health.[14,15] Fish are good bioindicators of heavy metals in aquatic ecosystems, because they are at the top of the aquatic food chain and accumulate high levels of metals from surrounding waters.[16,17] Therefore, the purpose of the present research was to investigate the distribution of the selected metals of Cd, Pb, Cr, Cu, and Zn in the muscle, gill, and liver of three fish species from the Sirvan River, Kermanshah Province, Iran. This study was conducted in order to assess the potential health risk of the intake of these heavy metals through the consumption of the edible parts of fish and also to determine the safe dietary intake level of these metals.

Materials and Methods

Fish samples were collected from random catches in Sirvan River during November to December, 2014. Fish species were carried to the laboratory in a thermos flask containing ice. In this study, the three fish species of common carp (Cyprinus carpio) (n = 20), tuwini (Capoeta trutta) (n = 20), and Grass carp (Ctenopharyngodon idella) (n = 20) were investigated in terms of metal levels (Cd, Pb, Cr, Cu, and Zn) in the edible muscle, gill, and liver. In the laboratory, they were immediately dissected using a stainless steel dissection instrument and clean plastic gloves. Samples of muscle were separated from under the dorsal fin of fish without skin.[13]

Approximately 1 g wet weight of gill, liver, and muscle were dissected from each sample and washed with distilled water, and accurately weighed into 150-ml Erlenmeyer flasks. To each sample, 10 ml nitric acid (65%) was added. Samples were left overnight in order to digest slowly. Afterward, 5 ml perchloric acid (70%) was added to each sample.[1,13] Digestion was performed on a hot plate (sand bath) at 150 ^{o}C before dilution with 25 ml deionized water. The concentrations of Cd, Pb, Cr, Cu, Ni, Zn, and Fe were measured using inductively coupled plasma atomic emission spectroscopy (ICP-OES) (AMETEK Materials Analysis Division, Germany). The detection limits for Cd, Pb, Cr, Cu, and Zn, were, respectively, 0.01, 0.2, 0.07, 0.3, and 0.75 µg/g. Moreover, the mean recovery for Cd, Pb, Cr, Cu, and Zn were 98.7, 98, 97.4, 96.7, 94.5, 96.6, and 99.4 percent, respectively.

Statistical analysis was performed using SPSS Software (version 16, SPSS Inc., Chicago, IL, USA). The one-way analysis of variance (ANOVA) was used to verify significant differences in organ metal concentrations among the three fish species. The metal concentrations in organs were expressed as microgram per gram wet weight (ww). Values are given in mean ± standard deviation (SD).

Daily consumption limits of metal contaminated fish were calculated according to the following equation. [18-20]

$$CR_{lim} = (RFD \times BW)/C_m$$

Where CR_{lim} is maximum allowable fish consumption rate (kg/d), RfD is reference dose (10 for Cd, none set for Pb, 3 for Cr, 300 for Zn, 20 for Cu, and 360 for Fe µg/kg/day), BW is the consumer's body weight (kg), and Cm is measured concentration of chemical contaminant m in a given species of fish (mg/kg).[20]

The consumption limit is determined in part by the size of the meal consumed. A 0.227 kg average fish meal size was assumed.[18] The following equation was used to convert daily consumption limits to the

number of allowable meals per month.[20]

$$CR_{mm} = (CR_{lim} \times T_{ap})/MS$$

Where CR_{mm} is the maximum allowable fish consumption rate (meals per month), CR_{lim} is the maximum allowable fish consumption rate (kg/d), MS is the average fish meal size (0.227 kg fish/meal), and T_{ap} is the time averaging period (365.25 days/12 months = 30.44 days/month).

Hazard quotient (HQ) is the ratio of the estimated exposure dose of a contaminant to its RfD. The HQ can be calculated with the following equation:[19-20]

$$HQ = [(MCC \times CR)/BW]/RFD$$

Where HQ is the health risks through consumption of fish by the local inhabitants, RfD is reference dose (10 for Cd, none set for Pb, 3 for Cr, 300 for Zn, and 20 for Cu µg/kg/day), BW is the consumer's body weight (kg), MCC is measured concentration of chemical contaminant m in a given species of fish (mg/kg), and CR is the average consumption rate (0.003 kg fish/meal). A target hazard quotient`t (THQ) below 1 means the exposure population is unlikely to experience evident adverse effects.[20] Acceptable daily and weekly uptake of heavy metals were calculated with the following equation:[20]

$$DI = C_m \times IR$$

where DI is the daily intake, C_m is the concentration of metal m in fish (µg/g) and IR is the intake rate.

Results and Discussion

The heavy metals concentrations in the muscle, gill, and liver of C. carpio, C. trutta, and C. idella are presented in figures 1 to 6. The results of the present research indicated that the metal levels in the liver and gill were higher than the muscle. Moreover, the results of statistical analysis showed that the levels of metals in organs of the three fish species were not significant (P > 0.05) (Table 1). The THQs in the three species of fish were below 1 (Table 2).

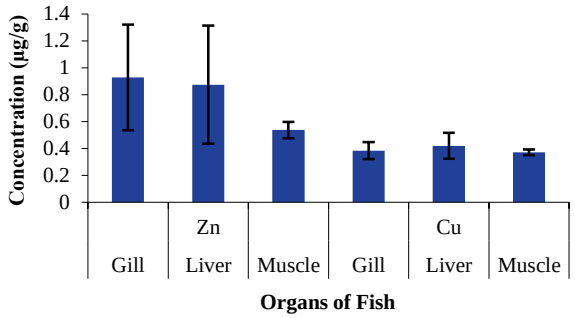

Figure 1. Cu and Zn concentrations (µg/g) in organs of Cyprinus carpio

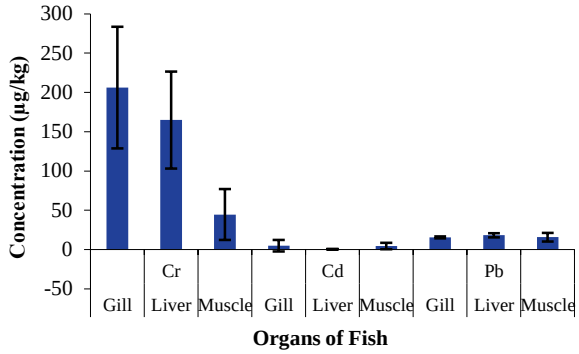

Figure 2. Cd, Pb, and Cr concentrations (µg/kg) in organs of Cyprinus carpio

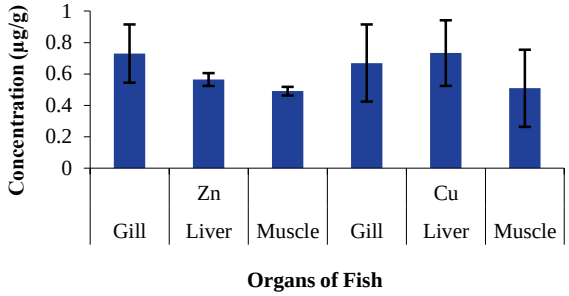

Figure 3. Cu and Zn concentrations (µg/g) in organs of Capoeta trutta

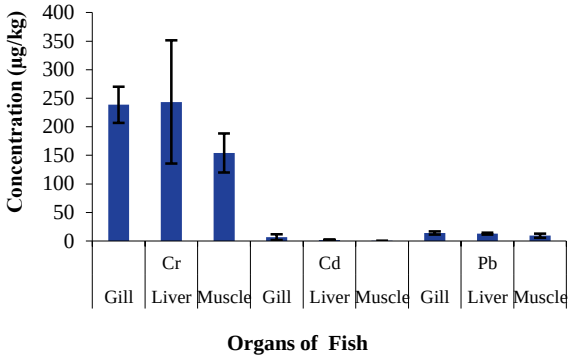

Figure 4. Cd, Pb, and Cr concentrations (µg/kg) in organs of Capoeta trutta

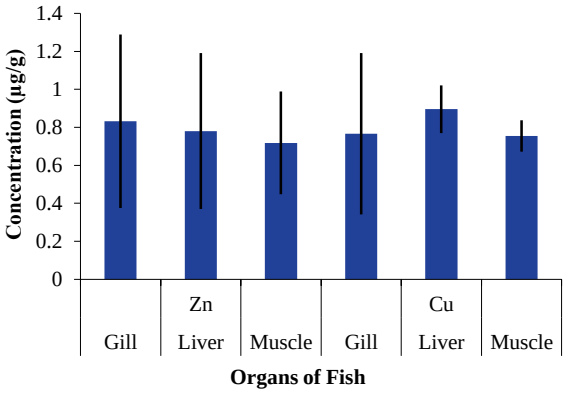

Figure 5. Cu and Zn concentrations (µg/g) in organs of Ctenopharyngodon idella

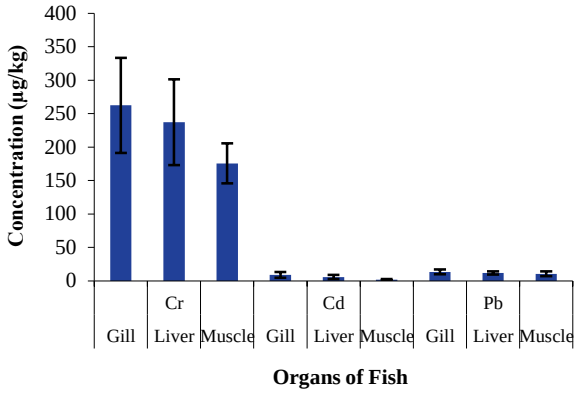

Figure 6. Cd, Pb, and Cr concentrations (µg/kg) in organs of Ctenopharyngodon idella

Table 1. Statistical analysis of metal levels in organs of the three fish species

Area/Fish species	Cd		Pb		Cr		Cu		Zn	
	One-way ANOVA		One-way ANOVA		One-way ANOVA		One-way ANOVA		One-way ANOVA	
	F-value	P	F-value	P	F-value	P	F-value	P	F-value	P
C. carpio	0.74	NS	0.52	NS	5.85	< 0.05	0.42	NS	1.15	NS
C. trutta	3.23	NS	2.38	NS	1.64	NS	0.73	NS	3.69	NS
C. idella	3.97	NS	0.52	NS	1.77	NS	0.27	NS	0.06	NS

P = significance level; NS = not significant

In this study, Cd, Pb, Cr, Cu, and Zn levels were higher in the gill and liver of the three fish species than the muscles. One reason for high concentrations of heavy metals in the gills and liver is the presence of large amounts of metallothionein (MT) protein and metal retention in these organs.[21] MTs, the multipurpose proteins, are low-molecular-weight proteins with many sulfhydryl groups which bind a variety of metals, such as Cu, Zn, and Cd, and show a strong affinity toward certain essential and non-essential metals.[22] In contrast, in muscle tissue, due to low metabolic activity, the accumulation of metals is low. Therefore, heavy metals uptake from aquatic environment via the muscle is much less important than the gills and liver. The liver also has an important role in contaminant storage, redistribution, and detoxification or transformation and acts as an active site of pathological effects induced by contaminants.[23] The liver tissue is highly active in the uptake and storage of heavy metals. Fish respond to heavy metal exposure by producing MT, particularly in the liver.[21] Thus, the liver tissues in fish are more often recommended as an environmental indicator of water pollution than any other fish organs.

Table 2. Hazard quotient, daily intake, and consumption rate indexes for the three species of fish in this study

Species	HQ	DI (µg/g)	CR (g)
C. carpio			
Zn	0.0007	10.90	39.1
Cu	0.0070	7.50	3.7
Cr	0.0060	0.80	4.7
Cd	0.0001	0.08	175.0
Pb	0.0006	0.30	46.6
C. trutta			
Zn	0.0007	10.10	42.8
Cu	0.0108	10.30	2.7
Cr	0.0220	3.10	1.3
Cd	0.00005	0.01	1400.0
Pb	0.0003	0.18	77.7
C. idella			
Zn	0.0006	14.60	29.2
Cu	0.0161	15.30	1.8
Cr	0.0375	3.50	8.0
Cd	0.0004	0.02	800.0
Pb	0.0004	0.20	70.0

HQ: Hazard quotient; DI: Daily intake; CR: Consumption rate

Cd, Pb, and Cr are toxic and non-essential metals and have no well-known role in the biological systems. They have been

recognized as environmental contaminants mainly due to their high toxicity at low concentrations.[1] Long term exposure to Pb and Cd may result in kidney and liver failure, and circulatory system and nerve tissue deficiency. Hanaa et al. have reported that exposure to high Pb concentrations may cause death or deterioration of the central nervous system, brain, and kidney.[5] Reported Pb levels in edible muscle of C. carpio (0.018 µg/g), C. trutta (0.012 µg/g), and C. idella (0.011 µg/g) were lower than the maximum acceptable concentrations (2.0 µg/g fresh weight of fish as seafood) reported by the European Commission (EC),[24] the UK Food Standards Agency,[25] Australian National Health and Medical Research Council (ANHMRC),[26] and Spanish legislation.[27] The concentrations of Cd obtained in C. carpio (0.004 µg/g), C. trutta, (0.001 µg/g), and C. idella (0.001 µg/g) edible muscles were lower than the maximum acceptable concentrations established by the EC (2001) (0.05 µg/g). Moreover, we found the Cd levels to be lower than the standards of the ANHMRC (2.0 µg/g), Western Australian authorities (5.5 µg/g),[28] and Spanish legislation (1.0 µg/g).[27]

The Food and Agriculture Organization of the United Nations (FAO)[29] has established a maximum permissible concentration of 30 µg/g for Cu (in this study, 9.8 µg/g for C. carpio, 12.8 µg/g for C. idella), and 30 µg/g for Zn (in this study, 19.5 µg/g for C. carpio and 15.4 µg/g for C. idella). The Cu and Zn concentrations in the edible muscle of both fish from Sirvan River were below the levels of concern for human consumption of toxic compounds. The Zn and Cu concentrations in the liver of C. carpio, C. trutta, and C. idella in the present study were lower than those in Hypophthalmichthys molitrix, Ctenopharyngodon idellus, and Megalobrama amblycephala in China,[2] and Labeo calbasu, Cirrhinus reba, and Rita rita in Chenab River, Pakistan.[30]

Hajeb et al. reported that the daily intake of heavy metals through food consumption is dependent on several factors such as the metal concentrations in food and amount of food consumed.[31] Health risk estimate levels measured for Cd, Pb, Cr, Cu, and Zn in all three fish species (Sirvan River) were below the international criteria in edible fish muscle for human protection.[32] In this study, to estimate the human health risk from consuming metal-contaminated fish, the estimated exposure doses were calculated for five metals. The maximum HQs determined for Cd, Pb, Cr, Cu, and Zn in all three fish species were 0.0004, 0.0004, 0.02, 0.01, and 0.0007, respectively. According to these results, THQ was lower than 1, and a THQ below 1 means the exposure population is unlikely to experience noticeable adverse effects.[20]

Conclusion

The health risks posed by exposure to heavy metals of Cd, Pb, Cr, Cu, and Zn, in local inhabitants of Kermanshah Province through the consumption of contaminated fish were investigated based on THQs. The results of the present study indicated that the THQ values are less than 1 for adults by consuming fish. The metal concentrations in the gill and liver were higher than in the muscles of the three species of fish. According to the results of this study, the levels of metals in the edible muscle of fish species in this study were below levels of concern for human consumption.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

Authors have acknowledged the Research Committee of Kermanshah Regional Water Co. for the financial support of this research project with the grant number KRSE-92089 and national code 7597-128602.

References

1. Majnoni F, Rezaei M, Mansouri B, Hamidian AH. Metal concentrations in tissues of common carp, Cyprinus carpio, and silver carp, Hypophthalmichthys molitrix from the Zarivar

Wetland in Western Iran. Archives of Polish Fisheries 2013; 21(1): 11-8.
2. Zhuang P, Li ZA, McBride MB, Zou B, Wang G. Health risk assessment for consumption of fish originating from ponds near Dabaoshan mine, South China. Environ Sci Pollut Res Int 2013; 20(8): 5844-54.
3. Couture P, Rajotte JW. Morphometric and metabolic indicators of metal stress in wild yellow perch (Perca flavescens) from Sudbury, Ontario: a review. J Environ Monit 2003; 5(2): 216-21.
4. Viarengo A. Heavy metals in marine invertebrates: mechanisms of regulation and toxicity at the cellular level. Aquat Sci 1989; 1(2): 295-317.
5. Hanaa M, Eweida A, Farag A. Heavy metals in drinking water and their environmental impact on human health. Proceedings of the International Conference for Environmental Hazard Mitigation; 2000 Sep 9-12; Cairo, Egypt.
6. Bruins MR, Kapil S, Oehme FW. Microbial resistance to metals in the environment. Ecotoxicol Environ Saf 2000; 45(3): 198-207.
7. Rainbow PS. Kenneth mellanby review award. trace metal concentrations in aquatic invertebrates: why and so what? Environ Pollut 2002; 120(3): 497-507.
8. Pan K, Wang WX. Trace metal contamination in estuarine and coastal environments in China. Sci Total Environ 2012; 421-422: 3-16.
9. Heidarieh M, Maragheh MG, Shamami MA, Behgar M, Ziaei F, Akbari Z. Evaluate of heavy metal concentration in shrimp (Penaeus semisulcatus) and crab (Portunus pelagicus) with INAA method. Springerplus 2013; 2(1): 72.
10. Sayyad NR. Heavy metal concentrations in different body part of crab, barytelphusa cunicularis from Godavari river. Int J Advan Agri Environ Eng 2014; 1(1): 51-3.
11. Astudillol LR, Yen IC, Bekele I. Heavy metals in sediments, mussels and oysters from Trinidad and Venezuela. Rev Biol Trop 2005; 53(Suppl 1): 41-53.
12. Alavian Petroody SS, Hamidian AH, Ashrafi S, Eagderi S, Khazaee M. Investigation of Body Size Effect on Bioaccumulation Pattern of Cd, Pb and Ni in the Soft Tissue of Rock Oyster Saccostrea cucullata from Laft Port. J Persian Gulf 2013; 4(14): 39-45.
13. Baramaki YR, Ebrahimpour M, Mansouri B, Rezaei MR, Babaei H. Contamination of metals in tissues of Ctenopharyngodon idella and Perca fluviatilis, from Anzali Wetland, Iran. Bull Environ Contam Toxicol 2012; 89(4): 831-5.
14. Daviglus M, Sheeshka J, Murkin E. Health benefits from eating fish. Comments on Toxicology 2002; 8(4-6): 345-74.
15. Patterson J. Introduction-Comparative Dietary Risk: Balance the Risk and Benefits of Fish Consumption. Comments on Toxicology 2002; 8(4-6): 337-43.
16. Vinodhini R, Narayanan M. Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (Common carp). Int J Environ Sci Technol 2008; 5(2): 179-82.
17. Bidar G, Pruvot C, Garcon G, Verdin A, Shirali P, Douay F. Seasonal and annual variations of metal uptake, bioaccumulation, and toxicity in Trifolium repens and Lolium perenne growing in a heavy metal-contaminated field. Environ Sci Pollut Res Int 2009; 16(1): 42-53.
18. Maleki A, Azadi NA, Mansouri B, Majnoni F, Rezaei Z, Gharibi D. Health risk assessment of trace elements in two fish species of Sanandaj Gheshlagh Reservoir, Iranian. Toxicol Environ Health Sci 2015; 7(1): 43-9.
19. Hosseini SM, Mirghaffari N, Mahbubi Sufiani N, Hosseini SV, Ghasemi AF. Risk assessment of the total mercury in Golden gray mullet (Liza aurata) from Caspian Sea. International Journal of Aquatic Biology 2013; 1(6): 258-65.
20. United States Environmental Protection Agency. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Risk assessment and fish consumption limits. 3rd ed. Washington DC: U.S. EPA Office of Water; 2000.
21. Kargin F. Metal concentrations in tissues of the freshwater fish Capoeta barroisi from the Seyhan River (Turkey). Bull Environ Contam Toxicol 1998; 60(5): 822-8.
22. Stankovic S, Kalaba P, Stankovic AR. Biota as toxic metal indicators. Environ Chem Lett 2014; 12(1): 63-84.
23. Licata P, Trombetta D, Cristani M, Naccari C, Martino D, Calo M, et al. Heavy metals in liver and muscle of bluefin tunA (Thunnus thynnus) caught in the Straits of Messina (Sicily, Italy). Environ Monit Assess 2005; 107(1-3): 239-48.
24. EC. Regolamento (CE) N. 466/2001 della commissione [Online]. [cited 2001]; Available from: URL: http://www.ittiologia.it/Normativa%20PDF/reg%20ce%20466-2001metalli%20pesanti.pdf
25. Cronin M, Davies LM, Newton A, Pirie JM, Graham T, Swan S. Trace metal concentrations in deep sea fish from the North Atlantic. Mar Environ Res 1998; 45(3): 225-38.
26. Rahman MS, Molla AH, Saha N, Rahman A. Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh. Food Chemistry 2012; 134(4): 1847-54.
27. Demirak A, Yilmaz F, Tuna AL, Ozdemir N. Heavy metals in water, sediment and tissues of

Leuciscus cephalus from a stream in southwestern Turkey. Chemosphere 2006; 63(9): 1451-8.
28. Plaskett D, Potter IC. Heavy metal concentrations in the muscle tissue of 12 species of teleost from cockburn sound, Western Australia. Australian Journal of Marine and Freshwater Research 1979; 30(5): 607-16.
29. Nauen CE. Compilation of legal limits for hazardous substances in fish and fishery products. Rome, Italy: Food and Agriculture Organization of the United Nations; 1983.
30. Waheed S, Kamal A, Malik RN. Human health risk from organ-specific accumulation of toxic metals and response of antioxidants in edible fish species from Chenab River, Pakistan. Environ Sci Pollut Res Int 2014; 21(6): 4409-17.
31. Hajeb P, Jinap S, Ismail A, Fatimah AB, Jamilah B, Abdul Rahim M. Assessment of mercury level in commonly consumed marine fishes in Malaysia. Food Control 2008; 20(1): 79-84.
32. Summers JK, Paul JF, Robertsonc A. Monitoring the ecological condition of estuaries in the United States. Toxicol Environ Chem 1995; 49(1-2): 93-108.

Journal of Advances in Environmental Health Research

Treatment of natural rubber industry wastewater through a combination of physicochemical and ozonation processes

Mohamadreza Massoudinejad[1], Mohsen Mehdipour-Rabori[2], Mohammad Hadi Dehghani[3]

1 Safety Promotion and Injury Prevention Research Center AND Department of Environmental Health Engineering School of Public Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2 Department of Environmental Health Engineering, School of Public Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran AND Environmental Health Engineering Research Center, Kerman University of Medical Sciences, Kerman, Iran
3 Department of Environmental Health Engineering, School of Public Health ,University of Medical Sciences, Tehran, Iran

Original Article

Abstract

In any type of rubber product manufacturing (including tires), the primary concerns are environmental. The aim of the present study was to survey a treatment combination of ozonation and physicochemical processes in the rubber industry. Wastewater samples were collected from the discharge unit of the rubber processing sewage system in Kerman Barez Tire Factory, Kerman, Iran. The wastewater samples used for chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), and oil and grease determinations were collected directly into bottles. After collection, samples were transferred to the laboratory for examination. The 2 methods of physicochemical process and ozonation process were used to treat wastewater. The study results suggest that the use of a chemical coagulation process with ferric chloride ($FeCl_3.7H_2O$) in the first stage of this study reduced COD by 37% of the original amount (0.56 g/l). The optimum dosage and pH range were 0.775 g/l and 6.5, respectively. When using $Al_2(SO_4)_3$, the COD reduction rate was 42%, and the optimum dosage and pH range were, respectively, 0.45 g/l and 6.5-7. After the ozonation process, COD was reduced by 70.75% and 90.6%. In accordance with these results and with respect to the high contamination load of this industry's wastewater and its many environmental hazards, the complete treatment of this industry's wastewater is crucial. One scientific and practical approach to wastewater treatment is the use of a combination of processes.

KEYWORDS: Aluminum sulfate, Coagulation, Ferric Chloride, Ozonation, Physicochemical, Wastewater, Treatment

Date of submission: 18 May 2015, ***Date of acceptance:*** 22 Jul 2015

Citation: Massoudinejad M, Mehdipour-Rabori M, Dehghani MH. **Treatment of Natural Rubber Industry Wastewater through a Combination of Physicochemical and Ozonation Processes.** J Adv Environ Health Res 2015; 3(4): 242-9.

Introduction

Industries are major sources of pollution in all environments.[1] Depending on the industry, different levels of pollutants are discharged into the environment directly or indirectly through the sewer outlet. In recent years, a rapid growth has been observed in industries due to the development of technology. Therefore, the volume of waste produced by different industries has also increased. The rubber industry produces environmental pollutants, which are highly objectionable, from natural rubber processing. The high concentrations of nitrogen and organic and inorganic loading in rubber wastewater pose serious threats to the environment.[2] Industrial wastewater includes employees' sanitary waste, production process discharge, wash waters, and contaminated water from heating and cooling and other operations.[3] To produce 20

Corresponding Author:
Mohammad Hadi Dehghani
Email: hdehghani@sina.tums.ac.ir

tons of rubber, a rubber factory produces, on average, 410,000 liters of effluent per day.[4] Environmental pollution caused by the daily discharge of about 80 million liters of untreated rubber effluent into near streams and rivers has been reported in Malaysia.[2,5,6] Without appropriate treatment, the discharge of rubber industry wastewater into the environment may have serious, dangerous, and prolonged consequences. Therefore, suitable technologies must be used to treat this wastewater.[7] Various methods for treating this type of waste exist in the world, the most important of which are biological,[8] aerobic,[9] anaerobic,[10] and physicochemical methods, and facultative ponds. Advanced methods include natural process,[11] electrochemical methods,[12] ozonation process followed by batch activated sludge, and methods combining physicochemical and biological methods (e.g., the gas injection technique and sludge process).[13] None of these studies, however, used the combination of physicochemical and ozonation processes.[8-13] The current study investigated the treatment efficiency of the combination of physicochemical treatment and ozonation process for chemical oxygen demand (COD) removal from rubber industry effluents.

Materials and Methods

In this study, a testing unit was conducted at a laboratory-scale in the Kerman Barez Tire Factory, Iran, to investigate the treatment efficiency of physicochemical and ozonation processes in a combined treatment method on rubber industry wastewater effluent.

The experimental stage was divided into 2 stages. In the first stage, experimental studies were conducted with a physicochemical treatment process in which effluent was treated through coagulation followed by flocculation using aluminum sulfate [alum, $Al_2(SO_4)_3$], and ferric chloride ($FeCl_3$). Different variables, including contact time, coagulant material dosage, and pH, were tested. In the second stage, ozonation was performed in the batch reactor as post treatment, and different variables, including contact time, ozone dosage, pH, and COD removal efficiency, were tested.

Rubber wastewater samples were collected from 9 production halls of a rubber wastewater processing factory in Kerman Province, Iran. Wastewater sampling was conducted at the output point, before discharge into the storage pond. After collection, the samples were fixed, transported to the laboratory, and immediately examined. First, each production unit was tested separately (9 units), and then, samples were mixed to make up 1 sample for the physicochemical treatment process.

The successful application of various coagulant materials in water and wastewater treatments has been reported in many studies.[14] Various types of natural and synthetic organic polymers have been used for the coagulation-flocculation process in wastewater treatment.[15] The main disadvantage of a physicochemical treatment process is the high volume of sludge it produces.[16]

Jar Tests

One well-known apparatus for selecting coagulant material for physicochemical wastewater treatment is the jar testing device, the results of which show treatment efficiency in terms of suspended matter and organic/inorganic matter removal.[17,18] Chemicals and coagulant materials are selected and optimum operating conditions (pH and exact amount of coagulant materials) are determined by means of jar testing.

Physicochemical experiments were carried out in a six-stirrer jar-test device (Phipps & Bird, USA). For the tests, 1000 ml of the sample was introduced into the jars. Then, the coagulant material was added by a beaker and the mixture was mixed rapidly (100 rpm) for 2 minutes. Subsequently, paddle velocity was decreased to 20 rpm for 20 minutes, and the flocculants were added into the tests in which ferric chloride ($FeCl_3$) and aluminum sulfate [alum, $Al_2(SO4)_3$] were used. Finally, the paddles were withdrawn so that the particles could settle for a 30-minute period.[19]

Rubber wastewater treated through physicochemical process is discharged into the ozonation reactor (50 liters) for treatment by ozone (Figure 1).

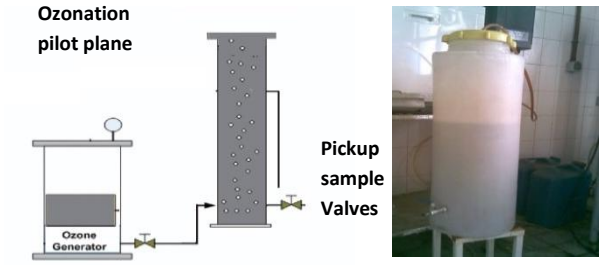

Figure 1. Schematic and photo of an ozonation pilot-scale

In all tests, COD, turbidity, pH, oil and grease content of the clarified water, and sludge volume after 30-minute sedimentation (V_{30}) were measured. COD and oil and grease contents were assayed using standard protocols given in section 5220 A.[20] In addition, pH was measured using a cation apparatus (Analytical Technology Inc., USA).

The ozone generator used in this study was a Compact Ozone Generator (OZONEUF, Model No. 6-5-11015, France Co., Ltd) (Figure 2). It was applied at various voltages and different ozone gas flow rates of 2-5 gr/hour. The ozone generation was determined by spectrophotometry using the standard potassium iodide (KI) absorption procedure.[21]

In this section of the treatment, ozonation was carried out in a 50-liter reactor fitted with a sand diffuser. A total of 6 samples were withdrawn periodically from the reactor by pickup sample valves. COD samples were collected at 7 contact times (15, 30, 45, 60, 90, 120, and 150 minutes). The initial value of COD was measured before the ozonation process was started.

The schematic and a photo of the ozonation pilot-scale are provided in figure 1.

Figure 2. Compact Ozone Generator

Results and Discussion

The tire and rubber industry has a great variety of uses for water and depends on it to cool its various types of equipment. Each use may have its own quality requirements.

Production Process in Kerman Barez Tire Factory:

The rubber tire manufacturing process includes the 11 steps of mixing, milling, extruding, calendering, bead making, cementing and marking inks processes, cooling and culture, tire-building, lubricating, curing, and tire finishing.[22]

All the steps mentioned above are associated with the consumption of large amounts of water which produces a volume of 2500 cubic meters of sewage per month in the studied factory.

Wastewater characterization:

The parameters analyzed were conductivity, pH, COD, oil and grease, and turbidity. The features of raw effluent are summarized in table 1.

Table 1. Characteristics of raw wastewater of Kerman Barez Tire Factory (separate production units)

No.	Unit production/Variable	pH	COD (mg/l)	TSS (mg/l)	Oil and grease (mg/l)
1	Old Banbury Mixer (hall 1)	9.9	26145	34900	26249
2	New Banbury Mixer (hall 2)	8.9	6391	1031	2925
3	Effluent of water boilers	10.3	3403	-	255
4	Effluent of cementing process	6.6	5146	77	7903
5	Effluent of makeup tube	8.5	3237	1328	853
6	Effluent of makeup tire	7.0	2830	1080	730
7	Effluent of reverse osmosis unit	8.0	270	300	0
8	Effluent of boiler blowdown	2.1	3901	31	3901
9	Effluent of curing process	6.6	3071	220	481

COD: Chemical oxygen demand; TSS: Total suspended solids

Table 2. Characteristics of mixed raw wastewater of Kerman Barez Tire Factory (final mixed samples)

No.	Parameters	Deal	Industrial effluent standard[23]
1	COD (mg/l)	5613	< 120 (not to exceed 400)
2	TSS (mg/l)	560	< 50 (not to exceed 150)
3	Oil and grease (mg/l)	5651	-
4	pH	8.3	5.5-9.0

COD: Chemical oxygen demand; TSS: Total suspended solids

The characteristics of mixed raw wastewater of Kerman Barez Tire Factory are presented in table 2.

The concentrations of COD, total suspended solids (TSS), and oil and grease were much higher than discharge effluent standards.

Wastewater characteristics after treatment through a 2-stage process:

First stage: Physicochemical Process

The relationship between pH and coagulant material dosage (ferric chloride and alum) is shown in figure 3.

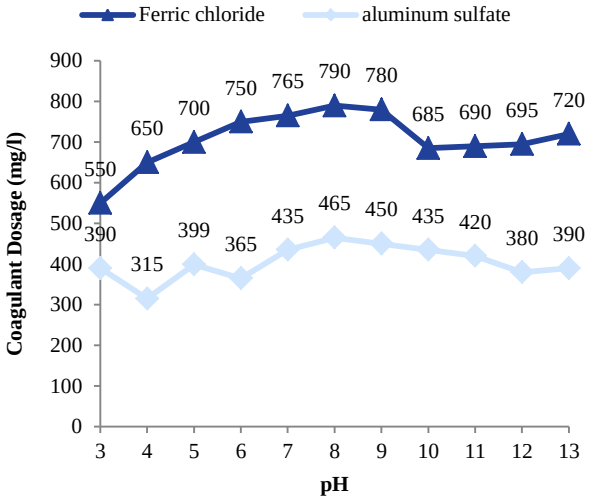

Figure 3. Relationship between pH and coagulant material dosage (ferric chloride and alum)

COD variation and removal efficiency in ozone pilot plane are shown in figure 4.

Second stage: Ozonation Process

In figure 5, the histogram of physicochemical treatment by ferric chloride and alum with and without ozonation is presented.

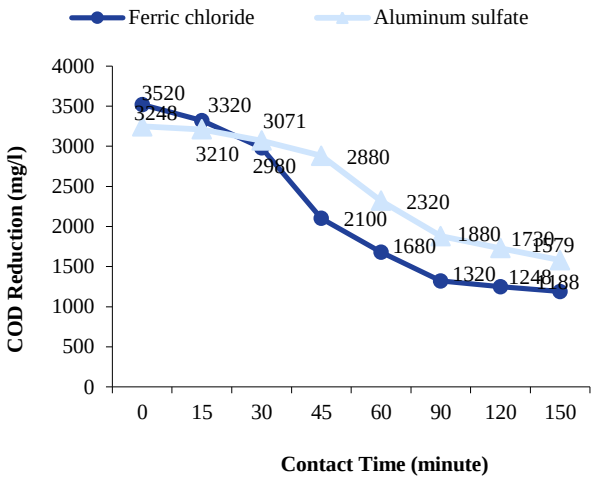

Figure 4. Chemical oxygen demand (COD) variation and removal efficiency in ozone pilot plane

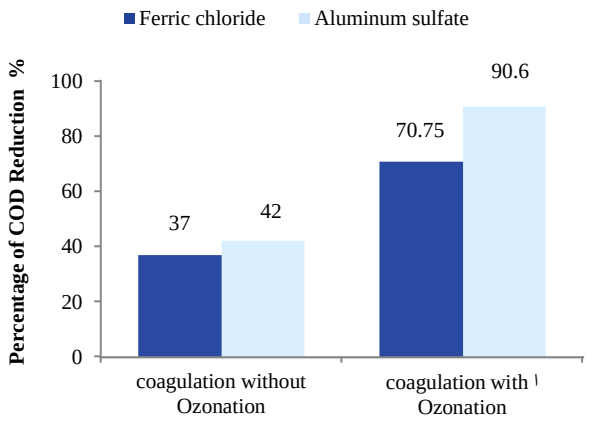

Figure 5. Physicochemical treatment by ferric chloride and alum with and without ozonation

The results of this study showed that, in the first stage, wastewater was treated through chemical coagulation with ferric chloride ($FeCl_3.7H_2O$) for a contact time of 30 minutes. In this stage, COD reduction was 37% of the original amount (5600 mg/l). The optimum dosage of chloride ($FeCl3.7H_2O$) and the pH range were 0.775 g/l and 6.5,

respectively. In the next stage of the physicochemical treatment with $Al_2(SO_4)_3$, the COD reduction rate was 42% of the original amount (5600 mg/l), and the optimum dosage and pH were 0.45 g/l and 6.5-7, respectively. In the second stage, after physicochemical treatment by $FeCl_3.7H_2O$ and $Al_2(SO_4)_3$, the wastewater was further treated by an ozonation process with 7 contact times (15, 30, 45, 60, 90, 120, and 150 minutes).

Ozone dosage was 5 gr/hour. The COD removal efficiency rates for the final effluent after the combined process are shown in figure 4 and are compared to the rates with and without ozonation. The removal efficiency of the COD parameter with the physicochemical process as a pretreatment was significantly higher than that with ozonation. This indicates that the combination process is better in terms of pollutant removal from the effluent of this industry. In the physicochemical stage of COD reduction, no significant difference in removal efficiency was observed with $Al_2(SO_4)_3$ (42%) and $FeCl_3.7H_2O$ (37%). In 2010, wastewater from the cheese industry was treated through coagulation-flocculation using $FeSO_4$, $Al_2(SO_4)_3$, and $FeCl_3$.[24] A ferric salt concentration of 250 mg/l resulted in a 40% to 60% reduction in COD. The optimum condition was found to be 3000 mg/l of $FeCl_3$ at pH of 5.6, resulting in a 76% removal of COD. Coagulation-flocculation using $Al_2(SO_4)_3$, $FeCl_3$, and aluminum polychorosulfate showed low efficiency for the removal of avian influenza virus subtype H_5N_1 and the swine-origin influenza A virus.[25,26]

A medical waste sterilization plant was pretreated with coagulation-flocculation using $FeCl_3$, ferrous sulfate, and $Al_2(SO4)_3$ by Ozkan et al.[27] Their results showed that a 60% COD removal and a high removal of suspended solids, nitrogen, and phosphorous were achieved through the application of 300 mg/l of $FeCl_3$ at pH of 10.[27] Paint manufacturing wastewater containing polyvinyl alcohol (PVA) was treated with alum.[28] The results showed that 80% COD removal was obtained with 2000 mg/l of both coagulants, whereas more than 90% removal was achieved for latex-based wastewaters with $FeCl_3$.[28] Chiavola et al. used alum, lime, and iron chloride as coagulants to treat olive mill wastewater.[29] Lime was selected as the best coagulant with 51% COD removal compared with alum and iron chloride. The results showed that the effluent was suitable for the subsequent biological treatment.[29] Kiril et al. found that the process of flocculation and coagulation and fission of acid improve oil biodegradation of olive oil mill wastewater.[30] Their results showed COD and phenol removal percentages of more than 67% and 72%, respectively.[30] Iron trichloride and aluminium polychloride were used as coagulation-flocculant material by Castrillon et al. to treat old landfill leachate.[31] The results of their study showed that 62% to 73% of non-biodegradable organic matter and more than 97% of turbidity and color were removed.[31] Samadi et al. studied the effects of different metal salt coagulants of polyaluminum chloride (PACl), alum, and ferrous on landfill leachate $Fe_2(SO_4)_3$ (1500 mg/l) which resulted in a higher than 71% COD removal at pH of 12.[32] Zazouli et al. used lime to treat fresh leachate.[33] The optimum concentration of lime as a coagulant was found to be 2.4 g/l and optimum pH was 9.5. Heavy metals and COD removal efficiencies of 79%-88% and 25%, respectively, were obtained.[33] Maranon et al. used $FeCl_3$, alum, PACl, and polyacrylamide polyelectrolytes for the coagulation-flocculation treatment of landfill leachate.[34] They found that ferric chloride concentration of 0.6 g/l at pH of 5-5.5 resulted in 73% COD, 98% color, and 100% turbidity removal.[34]

In another study, alum, $FeCl_3$, and ferrous sulfate showed the same performance for COD removal in the coagulation by precipitation (C/P) process, while for coagulation/dissolved air flotation (C/DAF),

the order of removal efficiency was alum > $FeCl_3$ > ferrous sulfate. Pre-ozonation was used before coagulation to investigate its effect on the formation, breakup, and regrowth of the flocs. Increased O_3 concentration showed an adverse effect on floc formation with limited regrowth of broken flocs.[35] The effects of the application of O_3 on the COD and color removal efficiencies of a textile industry were investigated by Avsar and Batibay.[36] O_3 was effective in removing COD and color and improved COD and color removal in addition to the current chemical treatments.[36] In 2010, Demin used ozone/biological activated carbon (BAC)/TiO_2 to treat phenolic wastewater.[37] The results showed that when phenol concentration was 0.1 g/l, the O_3-containing air flow rate was 0.05 m^3/hour, O_3 concentration was 3.58 mg/l, pH value was 7.5, and treatment time was 30 minutes. Moreover, the phenol removal rate was 99% and COD removal rate was 55%.[37]

Orta de Velasquez et al. investigated the effect of O_3 on dissolved organic matter during wastewater coagulation using alum.[38] Adding O_3 to the coagulant treatment enhanced the quality of the final effluent compared with conventional coagulation treatments.[38] Lafi et al. investigated the use of a combination of coagulation and advanced oxidation processes (AOPs) for the removal of organic pollutants from olive oil mill wastewater. The percentage of COD removal of coagulation with O_3 was a little lower than that with O_3/UV and H_2O_2/UV.[39] Hernandez-Ortega et al. reported combined electrocoagulation-ozonation to be a suitable pre-treatment for traditional biological processes and as a complete treatment for the discharge of industrial effluents into municipal sewers.[40] The combination of a biological treatment with the electrocoagulation-ozonation process led to a high-quality effluent.[40,41]

Conclusion

The combination of a physicochemical process with $Al_2(SO_4)_3$ as a pretreatment to the ozonation process can improve the efficiency of COD removal by up to 90.6%. This combined process was found to be very effective in removing the pollutants present in rubber industry wastewater. Moreover, it produced a final effluent which was low in suspended solids, clear, and odorless. However, the individual processes and the combined process (physicochemical and ozonation) were not sufficient to completely treat the highly polluted rubber wastewater. Thus, it is necessary to complete the treatment process with a method such as activated sludge. To reach industrial effluent standards, complementary processes such as activated sludge can be used.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

It is necessary to thank all those who participated in this research, especially the authorities of Barez Tire Factory in Kerman and the Department of Environmental Health Engineering, School of Public Health, of Shahid Beheshti University and Tehran University of Medical Sciences.

References

1. Asia IO, Akporhonor EE. Characterization and physicochemical treatment of wastewater from rubber processing factory. Int J Phys Sci 2007; 2(3): 061-97.
2. Babel S, Rungruang N. Treatment of natural rubber processing wastewater by combination of ozonation and activated sludge process. Proceedings of the International Conference on Environmental Research and Technology (ICERT 2008); 2008 May 28-30; Penang, Malaysia.
3. Emongor V, Nkegbe E, Kealotswe B, Koorapetse I, Sankwasa S, Keikanetswe S. Pollution Indicators in Gaborone Industrial Effluent. J Appl Sci 2005; 5(1): 147-50.
4. Mohammadi M, Man HC, Hassan MA, Yee PL. Treatment of wastewater from rubber industry in Malaysia. Afr J Biotechnol 2010; 9(38): 6233-43.
5. Hutagalung R. Use of carbohydrate residues in Malaysia. Proceedings of the 4th Symposium of the

International Society for. Tropical Root Crops held at CIAT; 1976 Aug 1-7; Cali, Colombia.
6. Tekasakul P, Tekasakul S. Present Status, Problems and Future Activities on Atmospheric Environmental Problems in South East Asian Developing Countries-Environmental Problems Related to Natural Rubber Production in Thailand. Earozoru Kenkyu 2006; 21(2):122-9.
7. Kannan A, Ranjithkumar P, Palpandi P. Status of common grazing and pasture land and livestock population in Madurai district: An environmental perspective. Zenith Int J Bus Economics Manag Res 2013; 3(8): 1-8.
8. Kantachote D, Torpee S, Umsakul K. The potential use of anoxygenic phototrophic bacteria for treating latex rubber sheet wastewater. Electron J Biotechnol 2005; 8(3): 314-23.
9. Anotai J, Tontisirin P, Churod P. Integrated treatment scheme for rubber thread wastewater: sulfide precipitation and biological processes. J Hazard Mater 2007; 141(1): 1-7.
10. Rosman NH, Nor Anuar A, Othman I, Harun H, Sulong Abdul Razak MZ, Elias SH, et al. Cultivation of aerobic granular sludge for rubber wastewater treatment. Bioresour Technol 2013; 129: 620-3.
11. Polprasert C. Constructed wetlands for wastewater treatment: principles and practices. In: Wong MH, Editor. Wetlands ecosystems in Asia: function and management. Philadelphia, PA: Elsevier; 2004. p. 285-310.
12. Vijayaraghavan K, Krishnan D, Ahmad Yazid AY. Electrolytic treatment of latex waste water. Desalination 2008; 219(1): 214-21.
13. Harunsyah MK, Sulaiman NM, Aroua MK. Ultrafiltration using gas sparging technique for treatment of natural rubber effluent [Online]. [cited 2003]; Available from: URL: http://repository.um.edu.my/id/eprint/64208
14. Afzal A, Pourrezaei P, Ding N, Moustafa A, Hwang G, Drzewicz P, et al. Physico-chemical processes. Water Environ Res 2011; 83(10): 994-1091.
15. Armeanu M, Cincu C, Zaharia C, Degeratu CN, Tudora RM. Active polymers for water treatment. Mater Plast 2010; 47(3): 274-7.
16. Bes-Pia A, Mendoza-Roca JA, Alcaina-Miranda MI, Iborra-Clar A, Iborra-Clar MI. Reuse of wastewater of the textile industry after its treatment with a combination of physico-chemical treatment and membrane technologies. Desalination 2002; 149(1): 169-74.
17. Aragones-Beltran P, Mendoza-Roca JA, Bes-Pia A, Garcia-Melon M, Parra-Ruiz E. Application of multicriteria decision analysis to jar-test results for chemicals selection in the physical-chemical treatment of textile wastewater. J Hazard Mater 2009; 164(1): 288-95.
18. Clark T, Stephenson T. Development of a jar testing protocol for chemical phosphorus removal in activated sludge using statistical experimental design. Water Res 1999; 33(7): 1730-4.
19. Sinha S, Yoon Y, Amy G, Yoon J. Determining the effectiveness of conventional and alternative coagulants through effective characterization schemes. Chemosphere 2004; 57(9): 1115-22.
20. Greenberg AE. Standard methods for the examination of water and wastewater. Washington, DC: American Public Health Association; 1981.
21. Clesceri LS. Standard methods for the examination of water and wastewater. Washington, DC: American Public Health Association; 1998.
22. Shechter H. Spectrophotometric method for determination of ozone in aqueous solutions. Water Research 1973; 7(5): 729-39.
23. Bradley LG, Pattanayak SK, Depro BM, Bingham TH. Economic Analysis of the Rubber Tire Manufacturing MACT [Online]. [cited 2000]; Avaiable from: URL: https://www3.epa.gov/ttnecas1/regdata/IPs/Rubber%20Tire%20Manufacturing_IP.pdf
24. Rivas J, Prazeres AR, Carvalho F, Beltran F. Treatment of cheese whey wastewater: combined coagulation-flocculation and aerobic biodegradation. J Agric Food Chem 2010; 58(13): 7871-7.
25. Lenes D, Deboosere N, Menard-Szczebara F, Jossent J, Alexandre V, Machinal C, et al. Assessment of the removal and inactivation of influenza viruses H5N1 and H1N1 by drinking water treatment. Water Res 2010; 44(8): 2473-86.
26. Liang Z, Wang Y, Zhou Y, Liu H, Wu Z. Stoichiometric relationship in the coagulation of melanoidins-dominated molasses wastewater. Desalination 2010; 250(1): 42-8.
27. Ozkan O, Mihciokur H, Azgin ST, Ozdemir O. Characterisation of medical-waste sterilisation-plant wastewater and a preliminary study of coagulation-flocculation treatment options. Water Sci Technol 2010; 62(2): 266-72.
28. Tunay O, Kocabas E, Olmez-Hanci T, Kabdasli I. Characterization and treatability of latex and pva based paint production wastewaters. Fresen Environ Bull 2010; 19(9): 1884-8.
29. Chiavola A, Farabegoli G, Rolle E. Combined biological and chemical-physical process for olive mill wastewater treatment. Desalin Water Treat 1987; 23(1-3): 135-40.
30. Kiril MB, Yonar T, Yalili KM, Kestioglu K. Pre-treatment studies on olive oil mill effluent using physicochemical, Fenton and Fenton-like oxidations processes. J Hazard Mater 2010; 174(1-3): 122-8.
31. Castrillon L, Fernandez-Nava Y, Ulmanu M, Anger I, Maranon E. Physico-chemical and biological

treatment of MSW landfill leachate. Waste Manag 2010; 30(2): 228-35.
32. Samadi MT, Saghi MH, Rahmani A, Hasanvand J, Rahimi S, Shirzad Sibani M. Hamadan landfill leachate treatment by coagulation-flocculation process. Iran J Environ Health Sci Eng 2010; 7(3): 253-8.
33. Zazouli MA, Maleki A, Izanloo H. Assessment of raw leachate characteristics and its pretreatment by lime. Asian J Chem 2010; 22(8): 6155-63.
34. Maranon E, Castrillon L, Fernandez-Nava Y, Fernandez-Mendez A, Fernandez-Sanchez A. Colour, turbidity and COD removal from old landfill leachate by coagulation-flocculation treatment. Waste Manag Res 2010; 28(8): 731-7.
35. Sam S, Yukselen MA, Zorba M, Gregory J. The Effect of Ozone on the Reversibility of Floc Breakage: Suspensions with High Humic Acid Content. Ozone: science & engineering 2010; 23(6): 435-43.
36. Avsar Y, Batibay A. Ozone application as an alternative method to the chemical treatment technique for textile wastewater. Fresen Environ Bull 2010; 19(12): 2788-94.
37. Demin Y. Treatment of toxic phenolic wastewater by advanced ozone oxidization [Online]. [cited 2010]; Available from: URL: http://en.cnki.com.cn/Article_en/CJFDTotal-CPPP201003024.htm
38. Orta de Velasquez MT, Campos-Reales-Pineda AE, Monje-Ramirez I, Noguez Y. Ozone application during coagulation of wastewater: effect on dissolved organic matter. Ozone: science & engineering 2010; 32(5): 323-8.
39. Lafi WK, Al-Anber M, Al-Anber ZA, Al-shannag M, Khalil A. Coagulation and advanced oxidation processes in the treatment of olive mill wastewater (OMW). Desalin Water Treat 2010; 24(1-3): 251-6.
40. Hernandez-Ortega M, Ponziak T, Barrera-Diaz C, Rodrigo MA, Roa-Morales G, Bilyeu B. Use of a combined electrocoagulation-ozone process as a pre-treatment for industrial wastewater. Desalination 2010; 250(1): 144-9.
41. Verma S, Prasad B, Mishra IM. Pretreatment of petrochemical wastewater by coagulation and flocculation and the sludge characteristics. J Hazard Mater 2010; 178(1-3): 1055-64.

JAEHR Journal of Advances in Environmental Health Research

Performance evaluation of the wastewater treatment plant of Pelareh Dairy Industry, Iran

Elnaz Mohebi-Fard[1], Mahdi Reyahi-Khoram[1], Soheil Sobhan-Ardakani[1]

1 Department of Environment, School of Basic Knowledge, Islamic Azad University, Hamadan Branch, Hamadan, Iran

Original Article

Abstract

Pelareh Dairy Industry (PDI) is located in the west of Iran. The aim of the present study was to assess the quality and quantity of PDI wastewater and compare the results with the regulations. PDI has a wastewater treatment plant that consists of sewage collection system, screening system, equalization tank, clarification tank, anaerobic system for pretreatment, activated sludge processing, disinfection, and solids drying beds. In this research, seven quality parameters, including chemical oxygen demand (COD), five-day biochemical oxygen demand (BOD_5), nitrate (NO_3), total suspended solids (TSS), phosphate (PO_4), temperature (T°c), and pH, were measured as qualitative variables. Thus, 20 samples were collected from influent and effluent zones. Wastewater samples were collected using random grab sampling during peak hours. Based on the results, the average (SD) COD concentration of the raw wastewater in wet season and dry season was 2152.22 (1384.00) and 1813.38 (518.33) mg/l, respectively. The results revealed that the removal efficiency of BOD_5, COD, and TSS at the studied plant was 89.22%, 88.79%, and 71.72%, respectively. Based on achieved results, the pollution load of PDI effluent wastewater was determined and presented. Based on the obtained results, the pollution load based on BOD_5 variable was 15.71 kg/day. The obtained results indicate that the treatment plant was not efficient enough to be considered as a treatment process for the removal of suspended solids and organic matter.

KEYWORDS: Environment, Industrial wastewater, Pollution, Wastewater

Date of submission: 12 May 2015, ***Date of acceptance:*** 22 Jul 2015

Citation: Mohebi-Fard E, Reyahi-Khoram M, Sobhan-Ardakani S. **Performance evaluation of the wastewater treatment plant of Pelareh Dairy Industry, Iran.** J Adv Environ Health Res 2015; 3(4): 250-7.

Introduction

Food processing is one of the most intensive water user industries and the volume of its wastewater is relatively high and dependent on the process details.[1] In the dairy industry, raw milk is processed into different products such as consumer milk, condensed milk, dried milk (milk powder), cheese, butter, yogurt, and ice cream. Unit operations in dairy industries that generate wastewater include disinfection and washing of equipment such as tanks, pipes, pasteurizers, centrifuges, homogenizers, pails, and etc. Depending on the capacity and type of the industry, raw industrial wastewater is highly polluted and contains high concentrations of organic matter such as carbohydrates, proteins, oil and grease, suspended solids, nitrogen, and a level of phosphorus. In addition, all of these substances contribute greatly towards the high values of five-day biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD).[2-8] It also has an unpleasant odor due to decomposition of some compounds such as casein which may have unsatisfactory effects.[5,9,10]

It is necessary to remember that almost all organic constituents of dairy waste are highly biodegradable.[11] In recent decades, many

Corresponding Author:
Mahdi Reyahi Khoram
Email: phdmrk@gmail.com

researchers have tried to determine the quantity of dairy wastewater generated by different industries. Based on the latest available information, total quantity of effluent produced in the industry is found to vary widely from company to company, and depends on the processing mode and its conditions.[10]

Kyrychuk et al. estimated that the amount of effluent wastewater generated by the dairy industry is in the range of 0.2 to 10 l of effluent per liter of pasteurized milk with the mean of about 2.5 l of wastewater per liter of pasteurized milk.[11] Karthikeyan et al. reported that the amount of wastewater produced annually by the mentioned industries was between 3.74 and 11.22 million cubic meters of wastewater per year, which means approximately one to three times the volume of milk processed.[12] Gulyas et al. confirmed that the volume of effluent wastewater produced for each cubic meter of processed milk is in the range of three to four cubic meters.[13]

Regarding the priority of environmental issues, it is necessary to adopt a positive approach to sustainable management of water, soil, and other finite resources and monitor industrial wastewater including dairy wastewater. On this basis, the aim of the present study was to investigate the quality and quantity of wastewater of Pelareh Dairy Industry (PDI) and also estimate the pollution load as part of environmental management policies in Iran.

Materials and Methods

PDI is located in Hamadan Province (Malayer Township) in the west of Iran, covering 2 hectares with three working shifts a day. The nominal capacity of the plant is approximately 55 tons of dairy product per day. These products are pasteurized milk, cheese, pizza cheese, and some types of yogurt. PDI has approximately 100 employees as permanent workers and workers' normal shifts are 8 hours a day, 7 days a week. The said factory has a wastewater treatment plant that consists of sewage collection system, screening system, equalization tank, clarification tank, anaerobic system for pretreatment, activated sludge processing, disinfection, and solids drying beds. Figure 1 provides a schematic flow diagram of PDI wastewater treatment facility.

This research project was conducted during 2014-2015. First, the general features of the study area and PDI were assessed using library and field visits. In this section, initial planning or pre-test was performed to assess the feasibility of the study, identify the site, investigate the problem, and determine the minimum sample size necessary to achieve a desired level of significance.

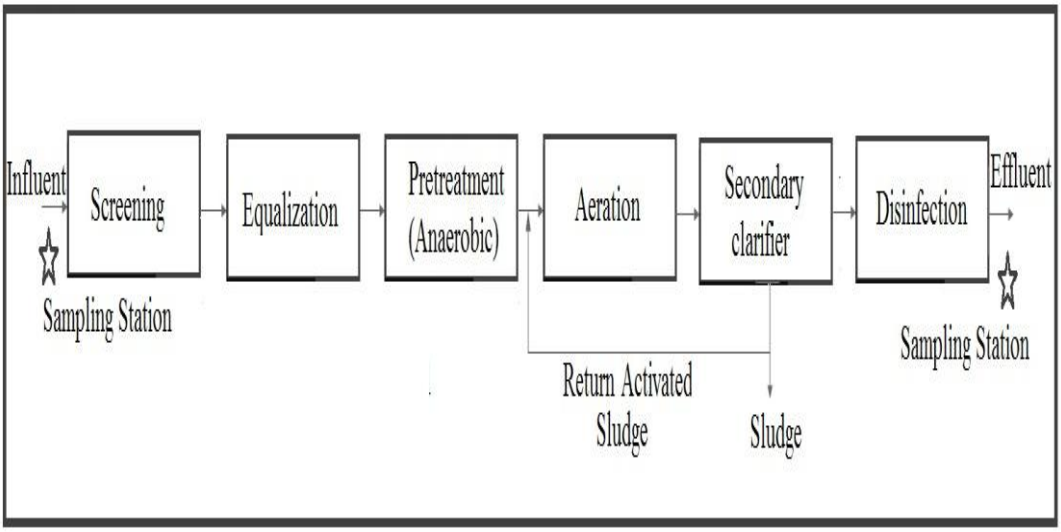

Figure 1. Flow diagram of Pelareh Dairy Industry wastewater treatment plant

Based on the water consumption rate, production volume, and number of workers, the total amount of wastewater, such as municipal wastewater and industrial wastewater, of the factory was obtained. Wastewater sampling was performed in two seasons (wet and dry). A computer random number generator was used to select 5 days in each season. During each sampling session, 20 samples were collected from influent and effluent zones. Wastewater samples were collected using random grab sampling during peak hours of activity and analysis was carried out as recommended by standard methods.[14]

In this research, 7 quality parameters, including COD, BOD_5, nitrate (NO_3), total suspended solids (TSS), phosphate (PO_4), temperature (T°c), and pH, were measured as qualitative variables to evaluate the quality of wastewater and pollution load. The efficiency of the wastewater treatment plant of the factory in terms of each parameter was determined in the following sections. The average concentration of each parameter was measured and compared against the standard. Microsoft Excel and SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA) were used for data analysis. Kolmogorov-Smirnov test was used for testing the distribution normality. The significance of all differences was tested using the one sample t-test.

Results and Discussion

The information obtained from PDI revealed that the amount of water consumed in the factory was 180 cubic meters per day. About 2% to 3% of this amount is related to municipal or human wastewater of the factory. A certain percentage of supplied water was commonly used for irrigation of the factory's green space. Based on the results achieved, the milk processing capacity of this factory was 70 cubic meters per day and the volume of wastewater generated by the industry was about 140 cubic meters per day. Under these circumstances, the amount of effluent wastewater generated by the industry was about 2 l of effluent per liter of pasteurized milk.

Kolmogorov-Smirnov test was applied to test the normality of distribution. The results of the Kolmogorov-Smirnov test indicated that the distribution of data was normal.

The quality of raw and treated wastewater of PDI in different seasons are presented in table 1. In many cases, the results were very similar in the wet season and dry season. Based on the said results, the average (SD) concentration of COD in the raw wastewater collected in the wet season and dry season was 2152.22 (1384.00) and 1813.38 (518.33) mg/l, respectively.

Table 1. Comparison of characteristics of raw and treated wastewater samples collected from Pelareh Dairy Industry in different seasons

Wastewater	Variable	Wet season			Dry season			P
		n	Mean	SD	n	Mean	SD	
Raw	COD	5	2152.22	1384.00	5	1813.38	518.33	0.62
	BOD_5	5	1128.00	714.22	5	954.00	281.74	0.63
	TSS	5	922.00	344.99	5	782.00	78.23	0.40
	NO_3	5	2.16	3.90	5	9.80	21.91	0.46
	PO_4	5	49.20	15.22	5	89.80	32.03	0.03*
	pH	5	6.26	2.67	5	6.08	2.59	0.91
	T	5	30.10	0.74	5	31.40	2.96	0.39
Treated	COD	5	210.18	64.04	5	234.14	129.19	0.72
	BOD_5	5	114.60	34.36	5	109.80	67.57	0.89
	TSS	5	328.00	38.34	5	154.00	49.79	0.01*
	NO_3	5	12.08	11.05	5	1.42	1.94	0.10
	PO_4	5	38.60	10.45	5	29.40	5.31	0.12
	pH	5	7.47	0.33	5	7.38	0.19	0.61
	T	5	28.60	1.29	5	30.30	0.67	0.04*

* Significant; $P < 0.05$; COD: chemical oxygen demand; BOD_5: Five-day biochemical oxygen demand; NO_3: Nitrate; TSS: Total suspended solids; PO_4: Phosphate; T°C: Temperature

Table 2. Quality of raw and treated wastewater samples collected from Pelareh Dairy Industry

Wastewater	Variable	n	Mean	SD	Minimum	Maximum
Raw	COD	10	1982.80	1001.31	1218.50	4592.90
	BOD_5	10	1041.00	520.00	720.00	2400.00
	TSS	10	852.00	247.11	660.00	1500.00
	NO_3	10	5.98	15.37	0.00	49.00
	PO_4	10	69.50	31.89	31.00	145.00
	pH	10	6.171	2.48	4.20	10.71
	T	10	30.75	2.15	28.50	35.50
Treated	COD	10	222.16	96.95	139.30	460.60
	BOD_5	10	112.20	50.60	57.00	228.00
	TSS	10	241.00	100.82	110.00	380.00
	NO_3	10	6.75	9.36	0.00	28.00
	PO_4	10	34.00	9.20	22.00	51.00
	pH	10	7.42	0.26	6.90	7.75
	T	10	29.45	1.32	27.00	31.00

COD: chemical oxygen demand; BOD_5: five-day biochemical oxygen demand; NO_3: nitrate; TSS: total suspended solids; PO_4: phosphate; T°C: temperature

Since the concentrations of variables in the raw and treated wastewater in different seasons were similar, the data were reanalyzed without regard for the season and is presented in table 2. Based on the reanalyzed data, the average (SD) concentration of COD of raw and treated wastewater was 1982.80 (1001.31) and 222.16 (96.95) mg/l, respectively.

Regarding the purpose of the study, it was necessary to compare the results with the reference standard. Therefore, average values of several variables were compared to the approved standard for wastewater reuse in agricultural irrigation, artificial recharge, and disposal into rivers.

The results showed that there was a highly significant difference ($P < 0.05$) in mean pH level among the test groups, which was in the normal range, compared to the Iranian maximum permissible limits (Table 3). In terms of pH, the treated wastewater of PDI can be reused in agricultural irrigation or be disposed in surface water. Moreover, there was a highly significant difference ($P < 0.05$) in mean TSS level among the test groups, which was higher than the maximum permissible limits in Iran (Table 3). In terms of TSS concentration, the treated wastewater of the mentioned factory cannot be reused in agricultural irrigation or disposed in surface water and groundwater.

Table 3. Comparison of average wastewater quality parameters of Pelareh Dairy Industry effluent wastewater and Iranian Department of Environment standards

Effluent use	Factor	n	Mean	SD	Standard value	t	P
Agriculture	BOD_5 (mg/l)	10	112.20	50.60	100	0.76	0.465
	COD (mg/l)	10	222.16	96.95	200	0.72	0.488
	TSS (mg/l)	10	241.00	100.82	100	4.42	0.002*
	PH	10	7.42	0.26	(6-8.5)	11.36	0.001*
Artificial recharge	BOD_5 (mg/l)	10	112.20	50.60	30	5.14	0.001*
	COD (mg/l)	10	222.16	96.95	60	5.29	0.001*
	NO_3 (mg/l)	10	6.75	9.36	10	1.10	0.301*
	PO_4 (mg/l)	10	34.00	9.20	6	9.62	0.001*
	PH	10	7.42	0.26	(5-9)	29.54	0.001*
Discharge into surface waters	BOD_5 (mg/l)	10	112.20	50.60	30	5.14	0.001*
	COD (mg/l)	10	222.16	96.95	60	5.29	0.001*
	TSS (mg/l)	10	241.00	100.82	40	6.30	0.001*
	NO_3 (mg/l)	10	6.75	9.36	50	14.62	0.001*
	PO_4 (mg/l)	10	34.00	9.20	6	9.62	0.001*
	PH	10	7.42	0.26	(6.5-8.5)	11.27	0.001*

*Significant; $P < 0.05$; COD: chemical oxygen demand; BOD_5: five-day biochemical oxygen demand; NO_3: nitrate; TSS: total suspended solids; PO_4: phosphate; T°C: temperature; SD: Standard deviation

Table 4. Removal efficiency of important variables in Pelareh Dairy Industry wastewater treatment plant

Variable	Raw (influent) wastewater	Treated (effluent) wastewater	Removal efficiency$^{\Psi}$(%)
COD (mg/l)	1982.80	222.16	88.79
BOD_5 (mg/l)	1041.00	112.20	89.22
TSS (mg/l)	852.00	241.00	71.72
NO_3 (mg/l)	5.98	6.75	-12.87
PO_4 (mg/l)	69.50	34.00	51.08
pH	6.17	7.425	-20.32
T	30.75	29.45	4.23

$^{\Psi}$Removal efficiency: [initial concentration - final concentration]/initial concentration; *100%; COD: Chemical oxygen demand; BOD_5: Five-day biochemical oxygen demand; NO_3: Nitrate; TSS: Total suspended solids; PO_4: Phosphate; T°C: Temperature

Based on the results, there was a significant and nonsignificant difference in terms of mean BOD_5 and COD concentrations, respectively, among the test groups compared to the maximum permissible limits in Iran (Table 3). Under these circumstances, disposal of treated wastewater of PDI into the ground or surface water is not permitted.

In addition, there was a highly significant difference ($P < 0.05$) between the test groups in terms of mean PO_4 level; its concentration was higher than the maximum permissible limits in Iran (Table 3).

The mentioned wastewater may not be appropriate for disposal into surface water or groundwater, because the concentration of PO_4 was higher than the Iranian maximum permissible limits.

It should also be noted that there was a significant or nonsignificant difference in mean NO_3 concentration level among the test groups, compared to the maximum permissible limits in Iran (Table 3). In terms of NO_3 concentration in the treated wastewater of PDI, the discharging of the treated wastewater of PDI into the ground or surface water is permitted.

The average amount of some variables in influent wastewater and effluent at PDI wastewater treatment plant, and the removal efficiency are summarized in table 4. The results revealed that the removal efficiency of BOD_5, COD, and TSS at the studied plant was 89.22%, 88.79%, and 71.72%, respectively.

Due to the importance of pollution load assessment of industrial wastewater, this value was calculated in the present study. Based on the obtained results, the pollution load of COD, BOD_5, TSS, NO_3, and PO_4 was 31.10, 15.71, 33.74, 0.94, and 4.76 kg/day, respectively.

The present study was successfully conducted to evaluate the quantity and quality of PDI wastewater and also to determine the treatment efficiency of this wastewater treatment plant.

Analysis of wastewater quantity

The obtained results confirmed that the quantity of PDI wastewater was about 140 cubic meters per day. Indeed, the quantity of wastewater generated by PDI was about 2 l of effluent per liter of processed milk.

Few studies have been carried out on the quantity of dairy wastewater. Comparable results were obtained by Mahendraperumal Guruvaiah et al.[15] Their study investigated the quality and quantity of dairy industry wastewater and concluded that the mentioned industry generates 0.2-10 l of wastewater per litre of milk processed.[15] Another study performed by Briaoi and Granhen Tavares suggested that dairy industry wastewater generates up to 10 l of wastewater per litre of milk processed.[16] Furthermore, Gulyas et al. reported that the volume of effluent wastewater produced for each cubic meter of processed milk is in the range of 3 to 4 cubic meters.[13] It is therefore concluded that the amount of water used in the dairy factory is less than the amount used in similar plants.

Analysis of the wastewater quality

Some different parameters and results were analyzed and the findings are explained in

the following sections.

BOD5 and COD

The BOD_5 and COD removal efficiency in our study was 89.22% and 88.79%, respectively (Table 4). This finding was similar to the results of other researches. For example, Gorra et al. reported a BOD_5 removal efficiencies of higher than 90% for treatment of dairy industry wastewater in a continuous flow.[17] In another study performed in the dairy industry, Harush et al. performed the aerobic biodegradation and coagulation process for the removal of COD.[18] In the mentioned research, the removal percentage of COD was up to 87.43%.[18] Other investigations of behavior of ferric sulfate as coagulant in chemical treatment for the removal of organic matter from dairy industry wastewater showed COD removal efficiency of 77.3%.[19]

Nitrate

Because of the aeration system of the wastewater treatment plant, a major part of the ammonium present in the wastewater can be removed and converted to nitrite and nitrate through the nitrification process as given below.[20]

$$NH_4^+ + 1.5O_2 \xrightarrow{\text{Nitrosomonas}} NO_2^- + H_2O + 2H^+ \quad (1)$$

$$NO_2^- + 0.5O_2 \xrightarrow{\text{Nitrobacter}} NO_3^- \quad (2)$$

In this research, the nitrate concentration in the raw wastewater and treated wastewater was 5.98 and 6.75 mg/l, respectively, and a tenuous (12%) increase was observed in nitrate ion concentration in the effluent. This result is similar to other previous studies conducted elsewhere.[20]

Schaafsma et al. found a statistically significant increase in nitrate concentration (82%) during the treatment of dairy wastewater in a constructed wetland system.[21] Ghaly et al. reported that ammonium was oxidized into nitrite, and then, into nitrate in a hydroponic wastewater treatment system during plant growth and the concentrations were dependent on the type and quantity of seeds.[22] In the mentioned research, it was revealed that nitrite and nitrate concentrations in a hydroponic wastewater treatment system increased with time during germination period, and then, decreased during the plant growth period.[22]

TSS

TSS is a key measurement for wastewater and its treatment. In this research, TSS concentration in the effluent was on average 852 mg/l before treatment, whereas after physical and biological treatment, it was on average 241 mg/l and 71.72% reduction was observed. In the current study, however, TSS removal was relatively poor and had low contrast, but the results showed that the wastewater treatment plant of PDI has the potential to provide higher efficiency. A study in 2011 on dairy industry wastewater in Italy indicated that the removal efficiency of TSS by constructed wetland was 94.5%.[23] In a similar study, 93.85% TSS removal efficiency from dairy industry wastewater was achieved in a treatment plant consisting of screening chamber, oil and grease removal, equalization tank, neutralization tank, primary clarifier (PC), aeration tank (AT), and secondary clarifier (SC).[24] In another study, the removal efficiency of TSS from dairy industry wastewater by constructed wetland was reported to be 81%.[25]

Phosphate

In this research, phosphate removal was poor and affected by many shortage factors, such as escalation of costs of energy, materials, and labor. The results of this analysis can be compared with similar studies performed in other countries.[9,10,15,20,26-29] For example, Balamane-Zizi and Ait-Amar reported the low phosphate removal efficiency of only 41.4% for treatment of dairy industry wastewater.[26] In this research, the removal efficiency of phosphate was 25.35% after precipitation. The phosphate biologic elimination efficiency obtained in the study

by Salame et al. was 16.04% and the global elimination efficiency was 41.4%.[30]

Pollution Load

Assessment of pollution load is useful in monitoring wastewater discharge.[31] In the current study, pollution load was mainly measured in order to assess the actual impact of PDI wastewater on the environment. Similar studies have been performed on pollution load by Hosseini-Zare et al.,[32] Kaia Oras and Eda Grüner,[33] Hafizul Islam et al.,[34] and Riyahi Khoram et al.[1] among others. Frameworks, methods, and extensions used in these studies vary with the format of the survey, parameters, approaches, and resources.

Conclusion

The present study focused on the performance evaluation of PDI wastewater treatment plant and compared its effluent wastewater with permissible discharge standards in Iran. The results revealed that the treatment plant was not efficient enough to be considered as a treatment process for the removal of suspended solids and organic matter. It is clear that groundwater quality in the study area will be affected by the factory effluent. It seems that the PDI wastewater treatment plant requires technical upgrading via a complete sedimentation system and biological system before it can be used as an effective treatment plant. It is recommended that more emphasis be placed on the development, repair, and maintenance of electrical, mechanical, and process equipment. It is also recommended that more attention be paid to experiential training programs for wastewater treatment plant operators and managers to conserve energy and reduce pollution and waste.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

This research was carried out as a master's thesis at the department of environment and was financially supported by Islamic Azad University, Hamadan Branch, to which the authors' thanks are due.

References

1. Riyahi Khoram M, Safikhani M, Khezri SM. Review of Hosein Aabad Sugar Factory (HASF) wastewater and assessment of its pollution load. J Appl Sci Environ Manage 2013; 17(3): 395-400.
2. Pallavi N, Mahesh S. Feasibility study of moringa oleifera as a natural coagulant for the treatment of dairy wastewater. International Journal of Engineering Research 2013; 2(3): 200-2.
3. Kowalska E, Paturej E, Zielinska M. Use of Lecane rotifers for limiting Thiothrix filamentous bacteria in bulking activated sludge in a dairy wastewater treatment plant. Arch Biol Sci 2014; 66(4): 1371-8.
4. Bazrafshan E, Moein H, Kord Mostafapour F, Nakhaie S. Application of electrocoagulation process for dairy wastewater treatment. J Chem 2013; 2013: 1-8.
5. Pathak U, Das P, Banerjee P, Datta S. Treatment of wastewater from a dairy industry using rice husk as adsorbent: treatment efficiency, isotherm, thermodynamics, and kinetics modelling. Journal of Thermodynamics 2016; 2016: 1-7.
6. Andrade LH, Mendes FDS, Espindola JC, Amaral MCS. Nanofiltration as tertiary treatment for the reuse of dairy wastewater treated by membrane bioreactor. Sep Purif Technol 2014; 126: 21-9.
7. Mostafa AA. Treatment of cheese processing wastewater by physicochemical and biological methods. International Journal of Microbiological Research 2013; 4(3): 321-32.
8. Ravva SV, Sarreal CZ. Survival of Salmonella enterica in aerated and nonaerated wastewaters from dairy lagoons. Int J Environ Res Public Health 2014; 11(11): 11249-60.
9. Lamas Samanamud GR, Loures CC, Souza AL, Salazar RF, Oliveira IS, Silva MB, et al. Heterogeneous photocatalytic degradation of dairy wastewater using immobilized ZnO. ISRN Chemical Engineering 2012; 2012: 1-8.
10. Shinkar NP. Comparative study of various treatments for dairy industry wastewater. IOSR Journal of Engineering 2013; 3(8): 42-7.
11. Kyrychuk I, Zmievskii Y, Myronchuk V. Treatment of dairy effluent model solutions by nanofiltration and reverse osmosis. Ukrainian Food Journal 2015; 3(2): 280-7.
12. Karthikeyan V, Venkatesh KR, Arutchelvan V. A correlation study on physico-chemical characteristics of dairy wastewater. Int J Eng Sci Techno 2015; 7(2): 89-92.
13. Gulyas G, Fazekas B, Varga R, Karpati A. Biological purification of chemically pre-treated dairy

wastewater before discharge into a municipal sewage systems. Hung J Ind Chem 2015; 43(1): 45-8.
14. American Public Health Association. Standard methods for the examination of water and wastewater. Rochester, NY: Scholar's Choice; 2015.
15. Guruvaiah M, Narra M, Shah E, James J, Kurchania A. Utilization of dairy wastewater for pollutants removal and high lipid biomass production by a newly isolated microalgal strains chloromonas playfairii and desmodesmus opoliensis. Int J Appl Sci Biotechnol 2015; 3(4): 699-707.
16. BriaoI VB, Granhen Tavares CR. Scientific Note: Ultrafiltration of effluents from a dairy industry for nutrient recovery: Effect of pressure and tangential velocity. Braz J Food Technol 2012; 15(4): 176-83.
17. Gorra R, Freppaz M, Zanini E, Scalenghe R. Mountain dairy wastewater treatment with the use of a irregularly shaped constructed wetland (Aosta Valley, Italy). Ecological Engineering 2014; 73: 176-83.
18. Harush DP, Hampannavar US, Mallikarjunaswami ME. Treatment of dairy wastewater using aerobic biodegradation and coagulation. International Journal of Environmental Sciences and Research 2011; 1(1): 23-6.
19. Konieczny P, Ekner E, Uchman W, Kufel B. Effective use of ferric sulfate in treatment of different food industry wastewater. Acta Sci Pol Technol Aliment 2005; 4(1): 123-32.
20. Ghaly AE, Farag HA, Verma M. A hydroponic system for purification of anaerobically treated dairy manure and production of wheat as a nutritional forage crop. Am J Agric Biol Sci 2007; 2(3): 206-17.
21. Schaafsma JA, Baldwin H, Streb CA. An evaluation of a constructed wetland to treat wastewater from a dairy farm in Maryland, USA. Ecol Eng 1999; 14(1□2): 199-206.
22. Ghaly AE, Kamal M, Mahmoud NS. Phytoremediation of aquaculture wastewater for water recycling and production of fish feed. Environ Int 2005; 31(1): 1-13.
23. Mantovi P, Piccinini S, Marmiroli M, Marmiroli N. Constructed wetlands are suitable to treat wastewater from Italian cheese productions. Water Practice & Technology 2011; 6(3): 1-9.
24. Chaiudhari DH, Dhoble RM. Performance evaluation of effluent treatment plant of dairy industry. Current World Environment 2010; 5(2): 373-8.
25. Pachpute A, Sanjivan Mahadik SK. Use of constructed wetland for treatment of dairy industry waste water. International Journal of Innovative Research in Science, Engineering and Technology 2014; 3(4): 197-200.
26. Balamane-Zizi O, Ait-Amar H. Combined processes for phosphorus removal from a dairy plant wastewater: conditions influencing the chemical process. Environ Sci Technol 2009; 2(2): 112-9.
27. Ebrahimi A, Asadi M, Najafpour GD. Dairy wastewater treatment using three-stage rotating biological contactor (NRBC). Int J Eng 2009; 22(2): 107-14.
28. BriaoI VB, Tavares CR. Pore blocking mechanism for the recovery of milk solids from dairy wastewater by ultrafiltration. Braz J Chem Eng 2012; 29(2): 393-407.
29. Ghezzehei TA, Sarkhot DV, Berhe AA. Biochar can be used to recapture essential nutrients from dairy wastewater. Solid Earth 2014; 6(6): 1101-25.
30. Salame C, Aillerie M, Khoury G, Balamane-Zizi O, Ait-Amara H. Terragreen 2012: Clean energy solutions for sustainable environment (cesse)study of the simultaneous elimination of phosphates and heavy metals contained in dairy wastewater by a physical-chemical and biological mixed process; consequences on the biodegradability. Energy Procedia 2012; 18: 1341-60.
31. Sadhana C, Raj K. Water quality and pollution load of river Mandakini at Chitrakoot, India. Int Res J Environment Sci 2013; 2(6): 13-9.
32. Hosseini-Zare N, Gholami A, Panahpour E, Jafarnejadi A. Pollution load assessment in the soil and water resources: a case study in karun river drainage basin, southwest of Iran. European Online Journal of Natural and Social Sciences 2014; 3(3): 427-34.
33. Oras K, Gruner E. The estimation of the wastewater generation and pollution load by the branches of industry. Proceeding of the International Work Session on Water Statistics; 2005 June 20-22; Vienna, Austria.
34. Hafizul Islam M, Rahman M, Ashra FU. Assessment of water quality and impact of effluents from fertilizer factories to the Lakhya River. International Journal of Water Resources and Environmental Engineering 2010; 2(8): 208-21.

Journal of Advances in Environmental Health Research

Analyzing costs of collection and transportation of municipal solid waste using WAGs and Arc GIS: A case study in Tabriz, Iran

Sajad Mazloomi[1], Behnaz-Al-Sadat Vaez Madani[2], Mehdi Hosseini[3], Monireh Majlessi[4], Ali Amarlooei[1]

1 Department of Environmental Health Engineering, School of Health, Ilam University of Medical Sciences, Ilam, Iran
2 Department of Environmental Pollution, School of Environment and Energy, Islamic Azad University, Science and Research Branch, Tehran, Iran
3 Department of Environmental Health Engineering, School of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran
4 Department of Environmental Health Engineering, School of Public Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Original Article

Abstract

Waste collection and transportation is considered as the most costly stage of waste management, to the extent that more than 70% of the total waste management costs are related to this sector. The aim of this study was to analyze the cost of the waste collection and transportation system of the 5th district of Tabriz, Iran, in 2015. For this purpose, the maps of collection routes and locations of waste storage tanks were drawn using ArcGIS software. The waste collection and transportation operations were performed in 3 areas of municipal services of the study area in 8 specific directions, with a distance of about 68051.03 m during 2722.04 minutes. According to the GIS maps and outputs of WAGS software, the main cost of waste collection was related to supplying human resources and fuel charges. According to the results obtained from WAGs, the total daily and annual costs of waste collection were 37163.5 × 10^3 and 13564.7 × 10^6 internal rate of return (IRR), respectively. Moreover, the cost of solid waste transportation from collection point to the transfer station was estimated at about 500 × 10^3 IRR per 1 kilometer. Outputs of WAGs software show that 7 vehicles and 343 storage tanks were needed for collection and transportation of 26297 tons of generated waste in the study area.

KEYWORDS: ArcGIS software, Collection and Transportation, Tabriz City, WAGS software, Waste Management

Date of submission: 20 May 2015, ***Date of acceptance:*** 18 Jul 2015

Citation: Mazloomi S, Vaez Madani BA, Hosseini M, Majlessi M, Amarlooei A. **Analyzing costs of collection and transportation of municipal solid waste using WAGs and Arc GIS: A case study in Tabriz, Iran.** J Adv Environ Health Res 2015; 3(4): 258-65.

Introduction

The recent increase in production of different types of waste in various quantities and qualities is due to industrialization.[1-3] Preserving the health of human beings and the environment and also raising the efficiency in all stages of solid waste management are the cause of the consideration of new waste management systems in metropolises.[4,5] In this framework, policies and scientific strategies have been applied in order to develop programs and effective solutions for the collection, transportation, and disposal of solid wastes.[6] The main components of municipal solid waste management include production, storage, transportation, processing, recycling, and disposal which are connected, and thus, should be systematically linked so that a uniform unit can be used.[7] Since the unsanitary disposal of solid wastes results in adverse environmental health

Corresponding Author:
Behnaz-Al-Sadat Vaez Madani
Email: environment.vaez59@gmail.com

impacts and exorbitant economic costs,[8] the implementation of a strong management system, which has a significant role in the reduction of these shortcomings, is of great importance.[9,10] The efficiency of the waste management program should be based on health, economy, and environmental engineering aspects.[7,11] The most difficult part of solid waste management is the collection stage, especially in megacities. One of the most effective factors in waste management is the cost of waste collection and transportation which constitutes about 50% to 70% of total cost of the solid waste management program.[12,13] Through the application of plans, devices, and optimized management of the waste collection system, such costs can be drastically reduced.[11]

Tabriz city, the capital of East Azerbaijan Province, with a population of over 1,500,000 people (Census in 2006) and an area of 45,481 km^2 is situated in northwest Iran. The city is one of the largest cities in Iran and is situated at an altitude of 1340 meters above sea level, a latitude of 38.07° N, and a longitude of 46.28° E.[14,15] Former route maps and plans for Tabriz city solid waste management were based on trial and error tests which were based on personal experiences of the previous experts and managers. The most considerable portion of the cost of solid waste management is related to the collection and transportation stages, and the lack of any previous scientific plans for solid waste management in Tabriz. Therefore, in the present study, WAGs software (United Nations MAB) was applied with the aim of the optimization of the solid waste collection system in the studied area.

Materials and Methods

Tabriz has a steppe climate and low humidity with the average annual rainfall of 285 mm, and long and cold winters and mild summers due to its elevation above sea level.[16] According to the latest administrative divisions, Tabriz consists of 10 metropolitan districts; each district is, on average, composed of 2 or 3 areas.[14] The area of study in this research was the 5th district which covers 3 areas and is about 12.76 km^2 (Figure 1). This district is located in the east and northeast of Tabriz.

This study has been carried out in different steps. The first step was library study, in which information was collected regarding solid waste management system, waste collection and transportation system, general plan of WAGs and ArcGIS software (Esri, Redlands, California, USA), and their implementation method.

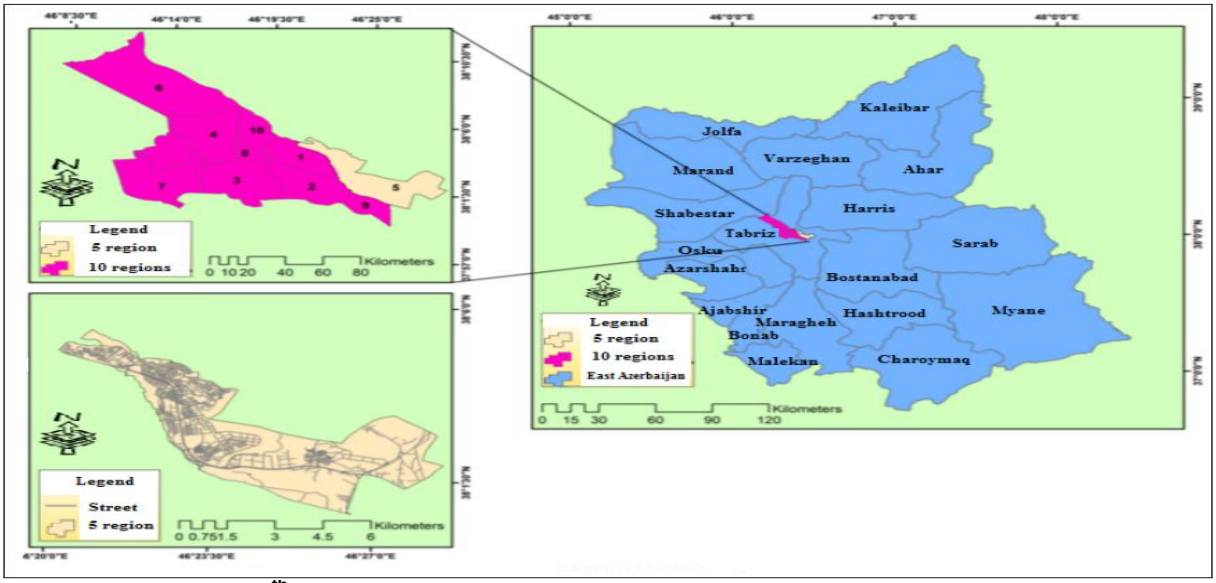

Figure 1. Map of the 5th district of Tabriz, East Azerbaijan Province

Table 1. Solid waste collection routes in the three areas of the 5th district of Tabriz

Routes of area 1	Routes of area 2	Routes of area 3
R* 1: Beheshti-Pardis	R 5: Zomorrod-Azad University	R 8: Jalali Cafe-Shahinshar
R 2: Ateshneshani-Misagh	R 6: Valiamr-Sheet 7	
R 3: Payam-Misagh	R 7: Talaeh-Missgh	
R 4: Fereshte-Dashtbani		

R*= Route

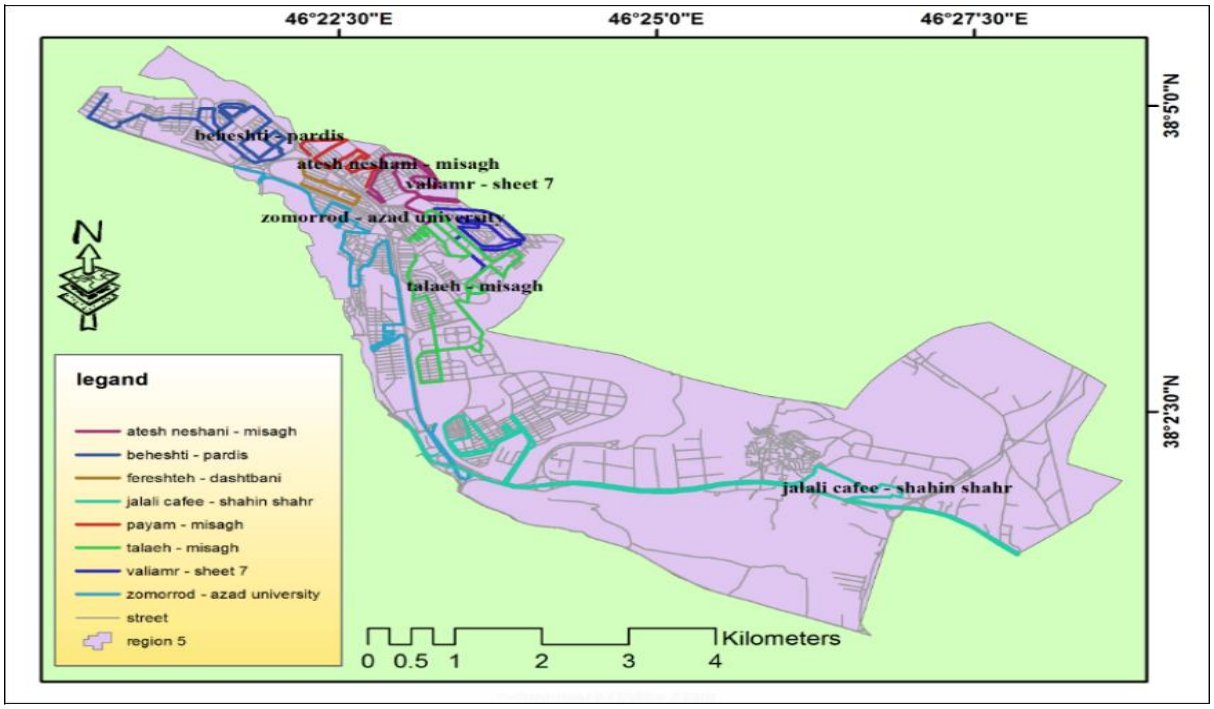

Figure 2. Map of the waste collection and transportation routes

In this stage, information was obtained about the study area, including Tabriz waste management organization, Tabriz Central Municipal Department of Planning and Development, and Department of Planning and Research and Department of Municipal Services of the 5th district of the municipality of Tabriz, from relevant bodies.

The second step was the implementation and execution of ArcGIS and WAGs software. The ArcGIS software was used to prepare and design the maps of collection routes. In general, the variables of WAGs software are classified into 5 categories of demographics information, quality and quantity of solid waste data, the location and characteristics of the study region, equipment used, and fees and taxes. The WAGS software requires the collection of 40 main parameters and 10 sub-parameters. The parameters are mainly related to the study area and solid waste, whereas sub-parameters are related to the vehicle and reservoirs of solid waste collection. In the final step, data obtained from the applied software were analyzed.

Results and Discussion

Based on the obtained information, total daily collected solid wastes in the 5th district of Tabriz (2015) was 72.10 tons. Solid wastes were collected 7 days per week in 3 shifts, and 7 mechanized vehicles were specified to collect all produced waste from the study area. Waste collection and transportation operations are carried out in 8 specific directions in 3 areas of the studied district (Table 1).

The waste collection and transportation routes were designed using ArcGIS software (Figure 2).

Table 2. Estimated distance from the collection point to the transfer station

Route number	waste collection routes	Distance (Kilometer)	Area
1	Beheshti-Pardis	7.56	Area 1
2	Ateshneshani-Misagh	5.68	
3	Payam-Misagh	3.20	
4	Fereshte-Dashtbani	1.81	
total		18.25	
5	Zomorrod-Azad University	11.25	Area 2
6	Valiamr-Sheet 7	5.24	
7	Talaeh-Misagh	10.22	
total		26.71	
8	Jalali Cafe-Shahinshahr	23.06	Area 3
total		23.06	

Determining the traveled distances

Using ArcGIS software, the total traveled distance by the waste collection vehicle from the collection point to the transfer station was found to be equal to 75 km. However, 68.05 km of this amount was related to the 8 routes of the solid waste collection and 6.95 km was related to the traveled distance from the collection area to the transfer station. Total traveled distances are presented in table 2 in detail.

Estimation of financial cost

WAGs software predicted that 7 vehicles and 343 storage tanks were required for collection and transportation of the 26297 tons of produced solid waste.

In addition, the total financial cost of waste collection and transportation was estimated to be 19334.5 × 10^6 internal rate of return (IRR) in 2015. As table 3 shows, the highest cost of waste collection was related to supplying human resources and fuel costs.

As the number of vehicles predicted by WAGs software was equal to the number of existing and active vehicles in the study area, only the cost of machinery depreciation in the intended year was considered for calculating solid waste collection costs. Based on information obtained from the Department of Municipal Services, the depreciation expenses of the active machinery were calculated as 7% (434.3 × 10^3 IRR) of the total machinery investment cost. Therefore, the cost of waste collection and transportation was estimated at 13564.7 × 10^6 IRR during the study year, which includes the costs of depreciation of machinery, human resources, and fuel and repair services, and other costs without investment in machinery costs.

Considering the obtained results from WAGs software and total traveled distance during the collection and transference operations, the cost of solid waste transportation to the transfer station was estimated at about 500 × 10^3 IRR per 1 kilometer (Table 4). Moreover, the total daily and annual costs were 37163.5 × 10^3 and 13564.7 × 10^6 IRR, respectively.

The total traveled distance, traveled time, and cost of solid waste collection and transportation operations in the study area were 68.05 km, 2722.04 minutes, and 34025.5 × 10^3 IRR, respectively. According to obtained initial data, the average speed of collection vehicles was 40 km/hour. The travel time of the vehicles was calculated by entering this data into ArcGIS software (Table 4).

As the number of vehicles predicted by WAGs software was equal to the number of existing and active vehicles in the study area, only the cost of machinery depreciation in the intended year was considered for calculating solid waste collection costs. Based on information obtained from the Department of Municipal Services, the depreciation expenses of the active machinery were calculated as 7% (434.3 × 10^3 IRR) of the total machinery investment cost. Therefore, the cost of waste collection and transportation was estimated at 13564.7 × 10^6 IRR during the study year, which includes the costs of depreciation of machinery, human resources, and fuel and repair services, and other costs without investment in machinery costs.

Table 3. Financial costs of solid waste collection and transportation in 2015 (in 106 IRR)

Investment in machinery	Human resources	Fuel costs	Cost of repairs	Other costs	Total cost
6204.100	8173.700	4267.400	623.700	65.600	19334.500

Considering the obtained results from WAGs software and total traveled distance during the collection and transference operations, the cost of solid waste transportation to the transfer station was estimated at about 500 × 10^3 IRR per 1 kilometer (Table 4). Moreover, the total daily and annual costs were 37163.5 × 10^3 and 13564.7 × 10^6 IRR, respectively.

The total traveled distance, traveled time, and cost of solid waste collection and transportation operations in the study area were 68.05 km, 2722.04 minutes, and 34025.5 × 10^3 IRR, respectively. According to obtained initial data, the average speed of collection vehicles was 40 km/hour. The travel time of the vehicles was calculated by entering this data into ArcGIS software (Table 4).

Estimation of waste collection and transportation costs

Currently, the waste collection and transportation operation for 3 areas is carried out through 8 specific routes. The related cost of every route for the travelled distance was calculated (Table 5).

According to the obtained results, the routes number 1, 2, 3, and 4 of area 1 cover 41, 31, 18, and 10%, respectively, of the total costs of collection and transportation. Waste collection and transportation operations of area 2 were carried out in 3 specific routes. The related cost of the 3 mentioned routes for the travelled distance was calculated at about 13358.200 × 10^3 IRR (Table 5). The related share of total cost of routes number 5, 6, and 7 was 42, 20, and 38%, respectively. Waste collection and transportation of area 3 was carried out in one route, and the related cost of this route was estimated at about 11534.4 × 103 IRR.

According to the obtained results of the three areas of municipal services in the studied area, 27, 39, and 34% of the total cost of waste collection and transportation, respectively, was allocated to areas 1, 2, and 3. Therefore, it seems that the priorities and percentage allocated to each of the areas, necessary measures, and appropriate planning should be taken into account in order to reduce costs.

Preparing the map of waste storage

One of the conventional problems of the waste collection and transportation system in the study area was the unsuitable situation of waste storage tanks which simultaneously increases the costs of the waste collection municipal services system. The number of storage tanks was calculated as 343 storage tanks for a total of 26297 tons produced solid waste using WAGs software. In addition, the longitude and latitude coordinates of the determined tanks were specified by ArcGIS on the exact location of the study area (Figure 3).

Table 4. Calculated distance, travel time, and the cost of waste collection and transportation system

Route number	Waste collection routes	Calculated distance (m)	Time (minutes)	Cost (× 10^3 IRR)
1	Beheshti-Pardis	7569.02	302.76	3784.510
2	Ateshneshani-Misagh	5682.90	227.31	2841.452
3	Payam-Misagh	3201.79	128.07	160.897
4	Fereshte-Dashtbani	1812.11	72.48	906.056
5	Zomorrod-Azad University	11251.75	450.07	5625.875
6	Valiamr-Sheet 7	5240.06	209.60	2620.031
7	Talaeh-Misagh	10224.50	408.98	5112.251
8	Jalali Cafe-Shahinshahr	23068.88	922.75	1153444.222

Table 5. The waste collection and transportation cost of every route of the study area

Area Number	Route number	Calculated distance (m)	Waste collection cost for calculated distance ($\times 10^3$ IRR)
1	1	7569.02	3784.5
	2	5682.90	2841.4
	3	3201.79	1600.9
	4	1812.11	906.1
	Total	18266	9132.9
2	5	11251.75	5625.9
	6	5240.06	2620
	7	10224.50	5112.3
	Total	26716.31	13358.2
3	8	23068.88	11534.4
	Total	23068.88	11534.4

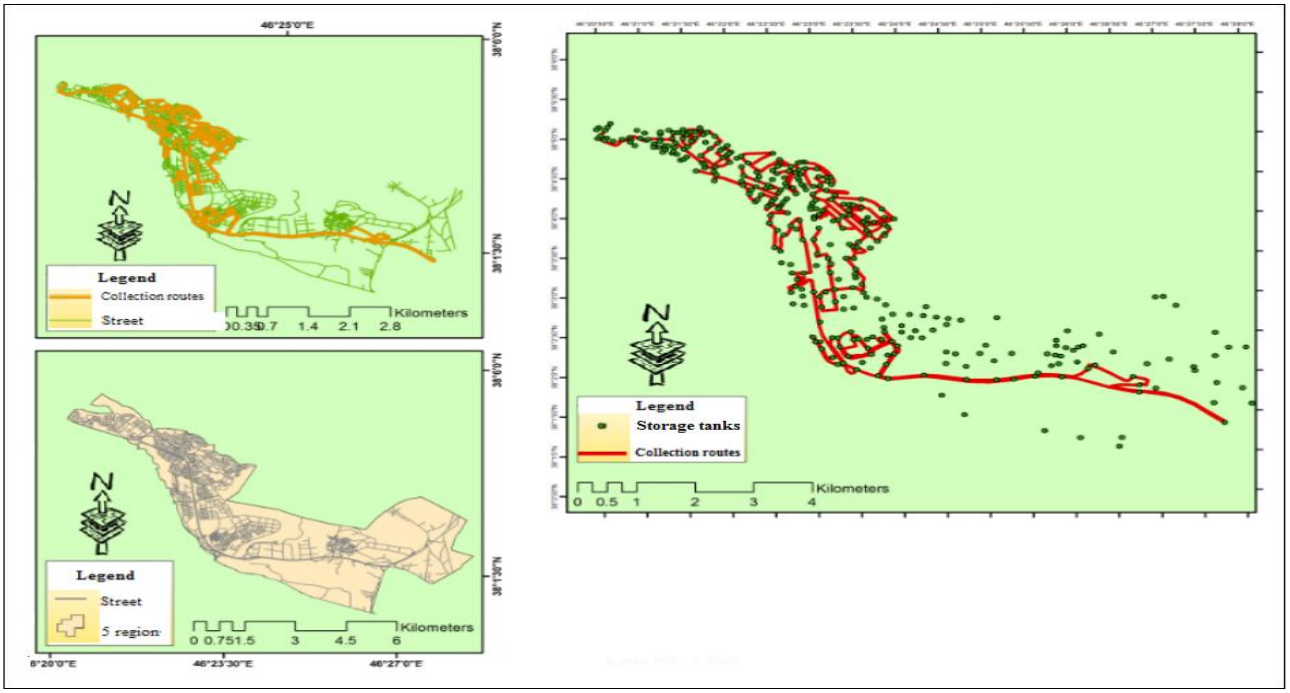

Figure 3. Map of waste storage tanks on determined locations

This study was carried out for the first time in the study area. The waste collection and transportation operations in 3 areas of the 5th district of municipal services are carried out in 8 specific routes, with length of about 68.051 km, in 2722.04 minutes. The cost for all travelled distances was estimated at 34025.5 × 10^3 IRR. It was estimated at about 500 × 10^3 IRR for each kilometer. However, because of management issues, other studies have focused on the price based on the weight of the waste.[17] The results showed that the highest and lowest waste collection and transportation cost belonged to route 8 in the 3rd area and route 4 in the 1st area, respectively. Similar to the results of this study, other studies have shown that with increasing time of collection, waste management costs will also increase.[18] Area 2, due to having the highest share of total costs of the study area, was specified as the critical area. Tavares et al. illustrated that recognition of collection routes using ArcGIS in Praia city and Santiago Island of Senegal can result in 8% and 12% fuel cost reduction.[19] Collection routes determination was based on personal experiences in the study area, which can lead to extra costs. The routes and storage tanks of the waste collection service were determined using

ArcGIS. Based on the map of waste storage tanks and obtained results, the storage tanks were not consistent with the condition of the district and did not practically cover the collection routes of all existing tanks in the district. This can increase waste collection and transportation costs. The results of this research showed that the main costs of collection and transportation of waste were related to human resources and fuel cost, which was consistent with similar studies.[13,20] Singh et al. reported that for cities with a population of over 300,000, capital cost of solid waste management was about $120 million per 1000 tons of daily capacity, whereas operating cost was estimated at $15 to $30 per ton.[21] The results of this study showed that the cost of collecting and transporting waste was an estimated 500 × 10^3 IRR (equivalent to 14.7$) per ton. However, the cost of residual waste collection for some European countries like France, Germany, and Spain was 60-71 €/ton.[22] Higher cost for provided services in European countries is due to higher average income in these countries.[21] Thus, for lower income regions, like the present study area, waste collection and transportation costs analysis should be considered more in organizing the financial structure and planning in the municipality for appropriate allocation of budget. By implementation of this program, economic and environmental strategies of collection operation can be executed and the costs reduced.

Conclusion

The present study attempted to analyze the collection and transportation costs of municipal solid waste of the 5th district of Tabriz city. For this purpose, WAGs and ArcGIS were applied. The results of this study showed that when ArcGIS software was used for routing the collection and transportation system, the routes were drawn more precisely and required less time. In this case, the operation cost of the collection and transportation system was reduced significantly. Primary studies of collection routes and waste storage maps revealed that collection routes did not cover all storage tanks in the study area. This indicates that poor layout and unprincipled storage tanks in the study area had permanently increased the cost of the collection and transportation system of solid waste.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

This research was supported financially by the Tabriz City Municipality. We thank all our colleagues at Tabriz City Municipality who supported this study.

References

1. Rathi S. Alternative approaches for better municipal solid waste management in Mumbai, India. Waste Manag 2006; 26(10): 1192-200.
2. Tchobanoglous G, Theisen H, Vigil S. Integrated Solid Waste Management: Engineering Principles and Management Issues. New York, NY: McGraw-Hill; 1993. p. 350-90.
3. Damani N, Koolivand A, Sadat M, Mahvi AH, Mazloomig S. Hospital waste generation and management in some provinces of Iran. Toxicol Environ Chem 2013; 95(6): 962-9.
4. Sharholy M, Ahmad K, Mahmood G, Trivedi RC. Municipal solid waste management in Indian cities - A review. Waste Manag 2008; 28(2): 459-67.
5. Giusti L. A review of waste management practices and their impact on human health. Waste Manag 2009; 29(8): 2227-39.
6. Srivastava PK, Kulshreshtha K, Mohanty CS, Pushpangadan P, Singh A. Stakeholder-based SWOT analysis for successful municipal solid waste management in Lucknow, India. Waste Manag 2005; 25(5): 531-7.
7. Manaf LA, Samah MA, Zukki NI. Municipal solid waste management in Malaysia: practices and challenges. Waste Manag 2009; 29(11): 2902-6.
8. Ngoc UN, Schnitzer H. Sustainable solutions for solid waste management in Southeast Asian countries. Waste Manag 2009; 29(6): 1982-95.
9. Boadi KO, Kuitunen M. Environmental and health impacts of household solid waste handling and disposal practices in third world cities: the case of the Accra Metropolitan Area, Ghana. J Environ Health 2005; 68(4): 32-6.

10. Agdag ON. Comparison of old and new municipal solid waste management systems in Denizli, Turkey. Waste Manag 2009; 29(1): 456-64.
11. Salhofer S, Wassermann G, Binner E. Strategic environmental assessment as an approach to assess waste management systems. Experiences from an Austrian case study. Environ Model Softw 2007; 22(5): 610-8.
12. Modak P. Municipal solid waste management: Turning waste into resources. Int j adv res technol 2010; 21.
13. Chalkias C, Lasaridi K. A GIS based model for the optimisation of municipal solid waste collection: the case study of Nikea, Athens, Greece. WSEAS Trans Environ Dev 2009; 10(5): 640-50.
14. Zarghami M, Akbariyeh S. System dynamics modeling for complex urban water systems: Application to the city of Tabriz, Iran. Resour Conserv Recy 2012; 60: 99-106.
15. Taghipour H, Mosaferi M. Characterization of medical waste from hospitals in Tabriz, Iran. Sci Total Environ 2009; 407(5): 1527-35.
16. Zarghami M, Abdi A, Babaeian I, Hassanzadeh Y, Kanani R. Impacts of climate change on runoffs in East Azerbaijan, Iran. Global Planet Change 2011; 78(3-4): 137-46.
17. Linderhof V, Kooreman P, Allers M, Wiersma D. Weight-based pricing in the collection of household waste: the Oostzaan case. Resour Energy Econ 2001; 23(4): 359-71.
18. Bhambulkar AV. Municipal solid waste collection routes optimized with arc GIS network analyst. Int J Eng Adv Technol 2011; 11(2): 202-7.
19. Tavares G, Zsigraiova Z, Semiao V, Carvalho MG. Optimisation of MSW collection routes for minimum fuel consumption using 3D GIS modelling. Waste Manag 2009; 29(3): 1176-85.
20. Koushki PA, Al-Duaij U, Al-Ghimlas W. Collection and transportation cost of household solid waste in Kuwait. Waste Manag 2004; 24(9): 957-64.
21. Singh GK, Gupta K, Chaudhary S. Solid Waste Management: Its Sources, Collection, Transportation and Recycling. Int J Env Sci Dev 2014; 5(4): 347-51.
22. Hogg D. Costs for Municipal Waste Management in the EU [Online]. [cited 2002]; Available from: URL: http://ec.europa.eu/environment/waste/studies/pdf/eucostwaste.pdf

JAEHR Journal of Advances in Environmental Health Research

Preparation of magnetic chitosan/Fe-Zr nanoparticles for the removal of heavy metals from aqueous solution

Maryam Khodadadi[1], Mohammad Hassan Ehrampoush[2], Amir Hossein Mahvi[3], Hadigheh Dorri[4], Lida Rafati[2], Ali Naghizadeh[4]

1 Department of Environmental Health Engineering, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2 Environmental Sciences and Technology Research Center AND Department of Environmental Health Engineering, School of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3 Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
4 Department of Environmental Health Engineering, School of Health, Birjand University of Medical Sciences, Birjand, Iran

Original Article

Abstract

Copper and hexavalent chromium are heavy metals that are harmful to human health. Natural adsorbent chitosan, due to its considerable properties such as the presence of functional groups of $-NH_2$ and -OH, non-toxicity, low cost, and biocompatibility, has gained much attention in pollutant removal. Therefore, in the present study, adsorption of chromium (VI) and copper (II) ions was conducted in a batch system using magnetic chitosan/Fe-Zr nanoparticles. In addition, the effect of different variables such as contact time, pH, adsorbent dose, initial concentration of heavy metals, and temperature were investigated. The results of the present study indicated that the highest efficiency in removal of chromium (VI) and copper (II) in pH of 4 were 99.52% and 97.72%, respectively. Moreover, adding 0.4 g of the composite at concentration of 5 mg/l can result in up to 97.43% removal of copper (II) and adding 1 g of this composite at the same concentration can result in more than 91% removal of chromium (VI). In addition, it was concluded that increasing the density of the heavy metals did not have a remarkable effect on the removal efficiency. The equilibrium related to adsorbent capacity and the amounts of nanoparticles were obtained using the plots of Langmuir and Freundlich adsorption isotherms for chromium (VI) and copper (II), respectively. The studied adsorbent had a high level of efficiency in the removal of heavy metals from aqueous solutions.

KEYWORDS: Magnetic Chitosan, Adsorption, Chromium, Copper

Date of submission:18 May 2015, ***Date of acceptance:***14 Jul 2015

Citation: Khodadadi M, Ehrampoush MH, Mahvi AH, Dorri H, Rafati L, Naghizadeh A. **Preparation of magnetic chitosan/Fe-Zr nanoparticles for the removal of heavy metals from aqueous solution.** J Adv Environ Health Res 2015; 3(4): 266-75.

Introduction

One of the most important toxic heavy metals is hexavalent chromium [Cr (VI)] and it enters water resources mostly through industrial effluents. Cr (III) is needed for natural carbohydrate metabolism of mammals, but Cr (VI) is highly toxic, carcinogenic, and mutagenic and is considered to be of high risk for humans and animals.[1] Copper is another heavy metal that is harmful to human health. Cu (II) is toxic and can increase complications like anemia, gastrointestinal discomfort, and risk of lung cancer.[2] The recommended limits of Cr (VI) and Cu (II) in drinking water are 0.05 mg/l and 1 mg/l, respectively.[3] There are various methods to remove heavy metals from water resources, including precipitation,

Corresponding Author:
Ali Naghizadeh
Email: al.naghizadeh@yahoo.com

solvent extraction, reverse osmosis, restoration, and adsorption. Among these methods, adsorption has been highly examined in the removal of heavy metals from aqueous solutions due to its simplicity and the availability of numerous adsorbing materials like nanomaterial, and carbonic, polymeric, and biological materials.[1,2] Chitin is an abundant natural compound made of mono-polysaccharide derived from the skeleton of insects, crustacea shells, the cell wall of fungi, and snails. Through the deacetylation process of chitin, a type of poly-amino-saccharides called chitosan is formed.[4]

Chitosan has attracted the attention of scholars due to its properties like hydrophilic quality, biological compatibility, biodegradability, non-toxicity, good adsorption, and broad application range. As chitosan contains high amounts of amine and hydroxyl groups, it has a high adsorption capacity in both physical and chemical removal of different types of metals like copper, chromium, silver, platinum, and lead. In fact, chitosan has the capacity of bonding with heavy metals up to more than 1 mmol/g, which is much higher than that of active carbon.[2,4] The tendency of chitosan to adsorb heavy metals is highly dependent on chitosan source, degree of deacetylation, nature of the given metal, amount of crystallization, amine content, and pH of the solution. Combining chitosan with other materials is also extensively applicable in removing heavy metals.[5] Chitosan in combination with alginate,[6] cellulose,[7] and clinoptilolite[8,9] has been utilized to remove copper.

To remove chromium, magnetic chitosan with a capacity of 69.40 mg/g and chitosan/montmorillonite with adsorption capacity of 41.67 mg/g were utilized.[10] However, to enhance the adsorption capacity of the adsorbents, much attention has been paid to the designing and synthesis of new adsorbents. Various types of hydrogels of magnetic chitosan have been prepared and utilized to treat contaminated water. For example, magnetic chitosan hydrogel bed/polyvinyl alcohol with fast coagulation have been successfully used in the removal of color.[11] Magnetic chitosan complex-coated Fe_3O_4 is used to remove Alizarin Red (AR) from aquatic environments.[12] Recently, Wang et al. have studied the application of magnetic chitosan composites (MCCs) in the removal of heavy metals and colors.[13] Compared to other adsorbents, processes using MCCs have a high capacity of adsorption, and rapid adsorption even in small amounts and short contact time.[1] The aim of the present study was to synthesize magnetic chitosan/Fe-Zr nanocomposite and investigate Cr (VI) and Cu (II) removal from aqueous solutions.

Materials and Methods

This experimental study was performed in Birjand University of Medical Sciences, Iran, (Environmental Chemistry Laboratory). The utilized materials were produced by Merck Company (Germany). The utilized chitosan had a deacetylation rate of 91.04% and was produced by Sigma-Aldrich Company (MO, USA). The utilized devices included Varian AA240 Atomic Adsorption Spectrometer (Varian Inc., Australia), PG T80+ UV/VIS spectrophotometer (PG Instruments Ltd, UK), fourier transform infrared spectroscope (FTIR) model Tensor 27 with spectrometry range of 400-4000 1/cm (Bruker Corporation, MA, USA), X-ray powder diffraction (XRD) model Panalytical Philips-XPERT-PRO (Philips, Amsterdam, Netherlands), scanning electron microscope (SEM) device model KYKY-EM3200, and vibrating sample magnetometer (VSM) (7400 series, Lake Shore Cryotronics Inc., Westerville, OH,USA).

Synthesis and Preparation of MCh/Fe-Zr Nanoparticles

Preparation of Magnetic Fe-Zr Nanoparticles

The most common method of producing magnetic nanoparticles is using chemical methods especially co-precipitation. In this method, to produce magnetic nanoparticles (Fe_3O_4), divalent and trivalent iron solutions are utilized along with an appropriate alkaline agent like soda or mixed ammonia in

controlled test conditions to produce a dark magnetic hydrophilic solution of water which contains magnetic particles with negative charge attached to the ions of alkaline material. In the present study, first Fe_2 and Fe_3 salts were dissolved with proportion of 1:2 in distilled water, and then, soda (1.5 mol) that was exposed to N_2 at 80 °C for 30 minutes was gradually added to it. To this solution, 30 CC of $ZrOCl_2$ 0.5 mol/l solution was added, and the mixture was stirred for 8 hours, and the resultant dark sediment was washed with deionizer water several times and dried at 70 °C.[13]

Preparation of Magnetic Fe_3O_4@Zr(OH)$_4$ Covered on the Surface of MCh/Fe-Zr Bead

First, 4 g chitosan was mixed in 400 ml citric acid with shaker for 1 hour. Then, 8 g magnetic $Fe_3O_4Zr(OH)_4$ nanoparticles was added to chitosan gel within 3 hours so that a slurry solution formed. NaOH 2 mol/l solution was added to this solution drop by drop with a glass syringe while stirring hard. The resulting macrosphere gel was allowed to settle in NaOH solution for 60 minutes, and then, the formed bead separated from NaOH was washed with deionizer water so that its pH was neutralized. In the next phase, the MCh/Fe-Zr synthesized adsorbent was dried using freeze dryer for 1 day.

Stock solutions (100 mg/l) of the chromium ion and copper under study were prepared by dissolving an appropriate weight of pure $K_2Cr_2O_7$ salt and copper nitrate in distilled water, respectively. Using HCL and NaOH solutions, pH was set at 0.1 mol. Afterwards, a certain dose of the adsorbent was added to the sample containing a certain density of metal ions, and the sample was mixed using a shaker for a certain time. To measure the density of the remaining Cr (VI), colorimetric analysis was conducted using a spectrophotometer at wavelength of 540 nm, and the remaining density of Cu^{2+} was measured using atomic adsorption device at wavelength of 324.8 nm. The studied parameters included pH (2-12), adsorbent rate (0.4-2 g/l), temperature (15.25 and 35 °C), concentration of heavy metals (0-10 mg/l), and contact time (0-720 minutes). All of the parameters were chosen according to previous studies. The balanced adsorption capacity of the adsorbent was measured using the following equation:

$$q_e = \frac{(C_0 - C_e)V}{m} \quad \text{(Eq. 1)}$$

whereq_e is the amount of adsorbed ions per unit mass of the adsorbent, C_0 is the initial density of metal ions in the solution in mg/l, C_e is the balanced density of metal ions in the solution in mg/l, and m is the weight of the adsorbent in g. The adsorption performance and capacity of the adsorbent in adsorbing the heavy metal ions from water solutions were measured using the Langmuir and Freundlich isotherm models. The Langmuir model proposes that adsorption occurs in a single layer or in a constant number of adsorption sites, all adsorption sites have equal energy, and the structure of the adsorbent is homogenous.[14] The Langmuir equation is provided bellow (Equation 2):

$$q_e = \frac{q_m K_L C_e}{1 + K_L\, C_e} \quad \text{(Eq. 2)}$$

Where q_e is the number of the balanced metal ions in mg/g, q_m is the maximum superficial adsorption capacity in mg/g, C_e is the balanced density of metal ions in the solution in mg/l, and KL is the Langmuir constant in l/mg that shows the superficial ed energy and integration tendency of joint sites. The Freundlich isotherm model describes adsorption in heterogeneous systems. This model is shown in the following equation (Equation 3):[15]

$$q_e = k_F C_e^{\frac{1}{n}} \quad \text{(Eq. 3)}$$

Where qe is the amount of the balanced metal ions in mg/g, C_e is the balanced density of metal ions in the solution in mg/l, K_F is the Freundlich constant which determines the adsorption capacity, and n is the Freundlich capacity that expresses the hardness or intensity of adsorption.

Results and Discussion

Fourier Transform Infrared Spectroscopy Spectrum

To observe the chemical structure of chitosan and the resulting changes in the composite of magnetic chitosan/Fe-Zr nanoparticles, FTIR technique was utilized. As indicated in figure 1, the presence of the down peaks proves the presence of the target functional groups in the structure of nanoparticles. Adsorbing peak of chitosan in 3459.9 was related to tensile proof of O-H, in 1415 to tensile proof of amide I and N-H, in 1339 to amide II and CH_3, 1272 to vibrating tensile proof of C-O carbonyl group, in 2993.2 to vibrating proof of C-H, in 570 to vibrating proof of Fe-O, in 1139.8 to tensile proof of C-O-C, and in 788 to vibrating proof of Fe-O available in chitosan. These changes in FTIR spectrum indicate that magnetic Fe_3O_4 nanoparticles have successfully been modified by chitosan.

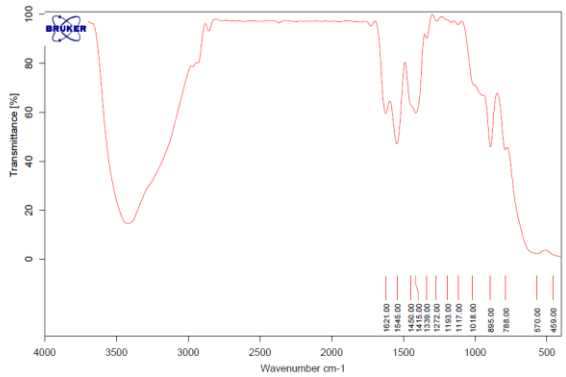

Figure 1. Fourier transform infrared spectroscope (FTIR) spectrum of composite of MCh/Fe-Zr nanoparticles

Examining the Magnetic Properties of Composite of MCh/Fe-Zr Nanoparticles

Vibration sample was examined at room temperature to examine the magnetic properties of MCh nanoparticles using magnetometer. Magnetic moment in magnetic field in 300 K for MCh is presented in figure 2. The magnetic curve indicates that MCh is super-paramagnetic and its magnetic saturation is 25.037. The synthesized adsorbent is dispersed and can be dispersed again. Moreover, these particles have good magnetic properties which refer to the potential application of magnetic adsorbent.

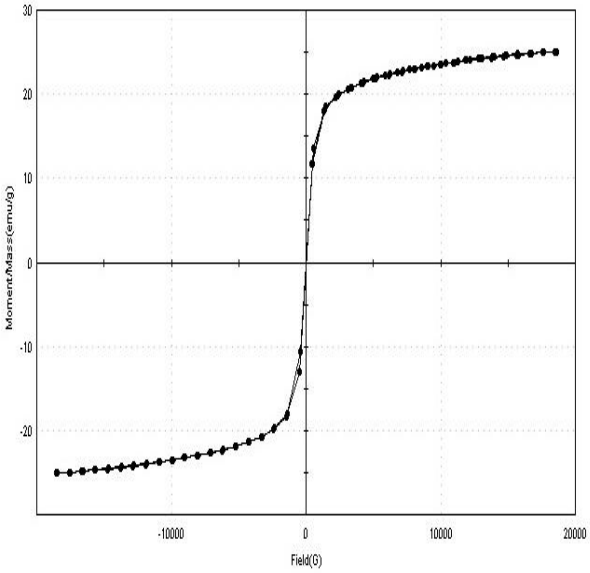

Figure 2.Vibrating sample magnetometer (VSM) curve of MCh/Fe-Zr nanoparticles

Examining the X-ray Diffraction Spectrum

The crystal structure of MCh composite is presented in figure 3. XRD patterns indicated certain peaks for MCh at 2θ, 30.313 (2 2 0), 35.707 (3 1 1), 43.401 (4 0 0), 53.85 (4 2 2), 57.41 (5 1 1), 63.05 (4 4 0), 75.64 (6 2 2), 32.155 (0 1 1), 36.613 (6 1 1), 57.33 (0 3 0), and 63.17 (18 1 1). This is in agreement with data presented by the Inorganic Crystal Structure Database (ICSD). XRD indicated that modification of chitosan-zirconium on magnetic nanoparticles has no effect on changing the phase of Fe_3O_4. The results also indicated high crystallization of MCh/Fe-Zr nanoparticles.

Examining Scanning Electron Microscope Images

The shape and size of MCh/Fe-Zr nanoparticles composite were determined using SEM. Figure 4 illustrates SEM images of the nanoparticles that have irregular shapes, spherical particles of Fe_3O_4 attached to the surface of the MCh-Zr particles with high density, and the size of the synthesized nanoparticles ranging from 31.9 to 140.4 nm.

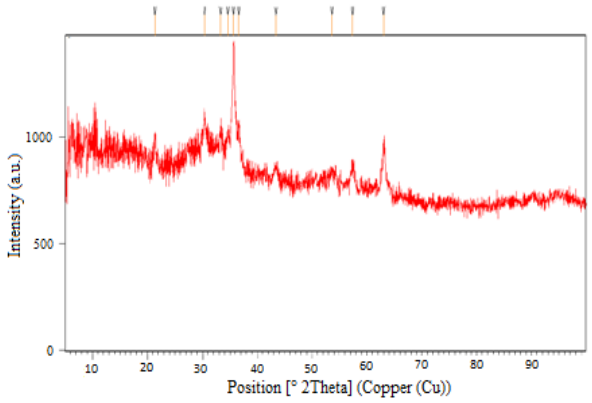

Figure 3. X-ray powder diffraction (XRD) spectrum of MCh/Fe-Zr nanoparticles composite

The Effect of Initial pH of the Solution on the Removal Rate of Cr (VI) and Cu (II)

The impact of initial pH of the solution in the range of 2-12 was investigated on the adsorption rate of metal ions of Cr (VI) and Cu (II) by the composite of MCh/Fe-Zr nanoparticles (Figures 5 and 6). It was observed that the highest adsorption rate for Cr (VI) ions using the proposed adsorbent in pH of 4 was 99.52% and for Cu (II) in pH of 9 was 97.72%.

Adsorption of heavy metal ions is highly reliant on protonation and non-protonation of amine and carboxylic groups available in chitosan nanoparticles.[16]As pH of the solution drops, amine groups existing in the composite of chitosan nanoparticles are protonated to different degrees. Therefore, the number of available sites for chelating of metal ions will decrease, which in turn causes the removal of electrostatic metal cations.[17] However, in high pH, the available ligands in the adsorbent like COO^- enhance the density of negative charge on the surface of the ligands. Therefore, electrostatic adsorption of metal ions with positive charge will increase on the surface of the ligands and the rate of adsorption will rise. Due to the concentration of OH^- ions in pH rates of higher than 9, deposition of metal ions is observed in the form of hydroxides and causes increase in the rate of adsorption.[18]

The effect of pH on adsorption of metal ions using chitosan and its derivatives has been investigated in different studies. Vasconcelos et al. reported pH of 6 as the optimal rate in experiments of adsorption of Cu (II) ions using cross-linked chitosan.[18] Alejandra Perez-Fonseca studied the removal of Cr (VI) using the composite of chitosan-covered agave fiber and high-density polyethylene (HDPE) wastes.[20]The optimal pH reported in their study was 4 and the maximum capacity of Cr (VI) adsorption was 200 mg Cr (VI)/g.[20]

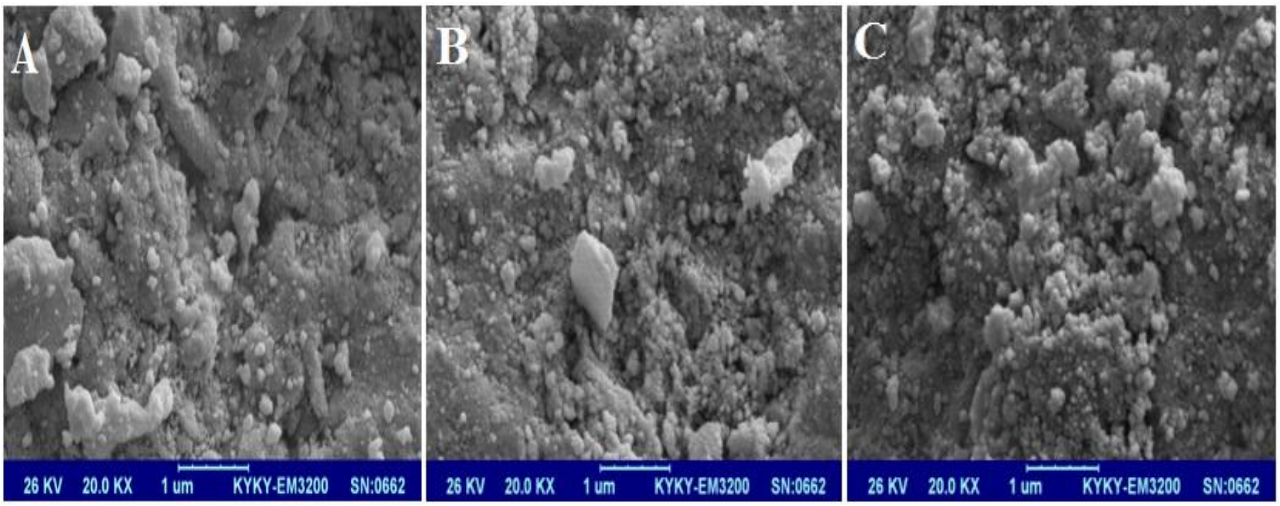

Figure 4. A: Scanning electron microscope image related to composite of MCh/Fe-Zr nanoparticles before adsorption of metal ions, B: scanning electron microscope image related to composite of MCh/Fe-Zr nanoparticles after adsorption of copper, C: scanning electron microscope image related to composite of MCh/Fe-Zr nanoparticles after adsorption of chromium

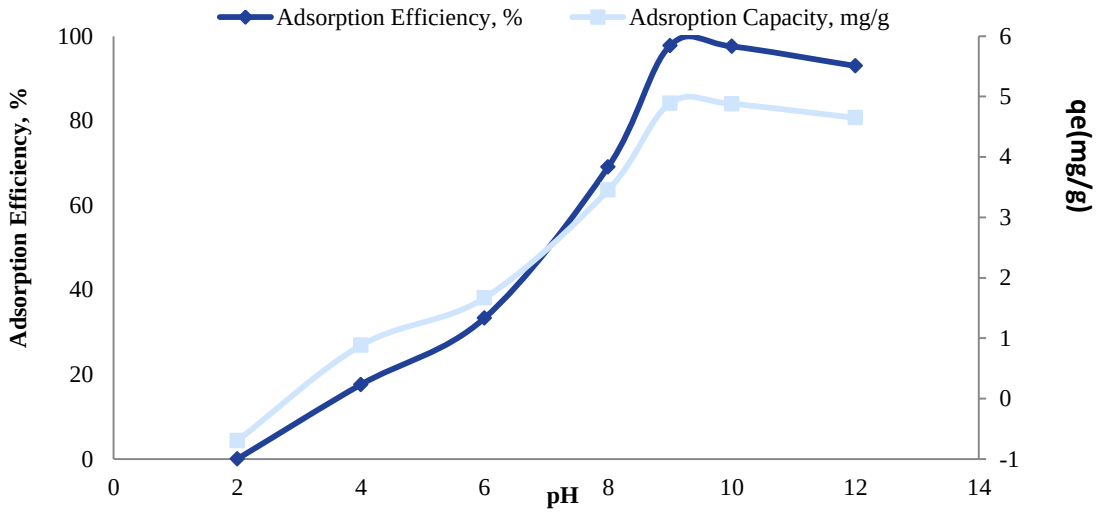

Figure 5. Comparison of the effect of pH changes on removal efficiency of copper using MCh/Fe-Zr nanoparticles
(Cu^{2+} = 5 ppm, speed = 300 rpm, time = 30 minutes, temperature = 25 ^{o}C, and adsorbent dose = 1 g/l)

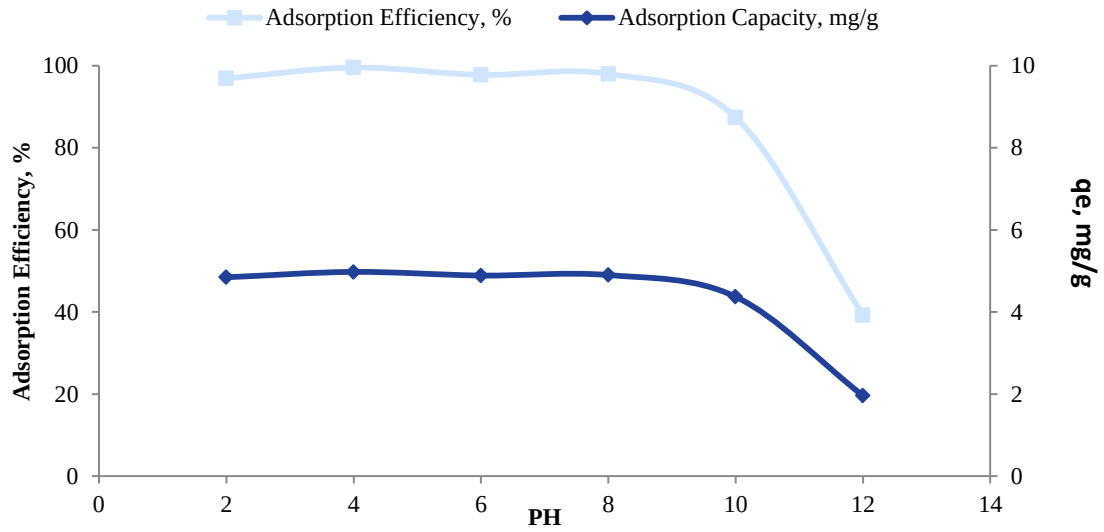

Figure 6. Comparison of the effect of pH changes on removal efficiency of copper using MCh/Fe-Zr nanoparticles (Cr6+ = 5 ppm, speed = 300 rpm, time = 30 minutes, temperature = 25 oC, and adsorbent dose = 1 g/l)

Tingyi Liu et al. investigated the removal of Cr (VI), Pb (II), Cd (II), and Cu (II) from industrial wastewater.[20]They concluded that the rate of Cr (VI) removal increases as pH decreases, and the removal efficiency of copper and lead increase as pH increases.[21]

Examination of the effect of the dose of MCh/Fe-Zr nanoparticles composite on removal rate of Cr (VI) and Cu (II) ions

Examination of the effect of adsorbent amount on the adsorption process of Cr (VI) and Cu (II) metal ions by the composite of MCh/Fe-Zr nanoparticles was carried out to determine the effective dose of the adsorbent. Considering the minimum amount of the adsorbent that has the highest rate of adsorption, the range of 0.4-2 g/l of adsorbent dosage was determined in the initial concentration of 10 mg/l. Figures 7 and 8 indicate the effect of adsorbent amount on the removal percentage of the ions. For Cr (VI), as the amount of the adsorbent increased from 0.4 to 2 g/l, the removal

percentage increased from 63.06% to 96.22% and the adsorption capacity decreased from 7.88 to 2.4 g/g, respectively. Yu et al. conducted a study of magnetic chitosan-Fe hydrogel in the removal of Cr (VI) and concluded that as the amount of magnetic Fe_3O_4 nanoparticles rises, the adsorption capacity drops.[22] They also found that the adsorption capacity of Cr (VI) after contact time of 30 minutes was 144.9 mg/g.[22] For Cu (II), the maximum removal efficiency obtained was 97.43 in adsorption dose of 0.4 g/l. As the amount of the adsorbent rises, the number of available adsorption sites increases, which enhances the amount of adsorbed metal. Decreased adsorption capacity and increased amount of adsorbent were mostly due to the fact that the adsorption sites, during the adsorption process and collection of particles, are not saturated in high amounts of adsorbent which results in surface area reduction.[24]In real applications, the minimum amount of the adsorbent that can respond to the requirements should be selected. In the study conducted by Dragan et al., the chitosan/clinoptilolite composite was used to remove copper ions, and the adsorbent amount in 100 mg/l was 2 g/l. under these conditions, the adsorption capacity was 719.39 mg/g.[8]

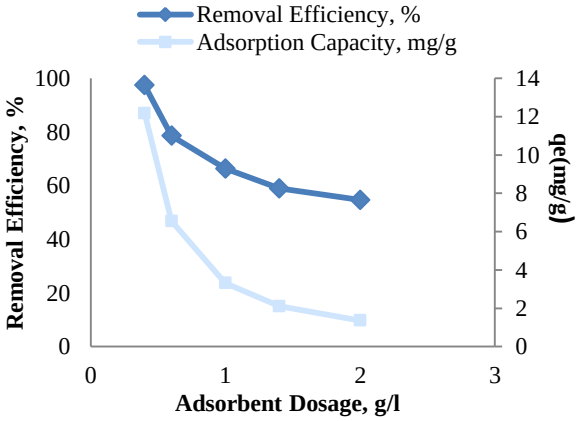

Figure 7. Comparison of the effect of adsorbent dose changes on the removal efficiency of copper using MCh/Fe-Zr nanoparticles
(density Cu^{2+}= 5ppm, speed = 30 rpm, time = 30 minutes, temperature = 25 °C, and pH = 9)

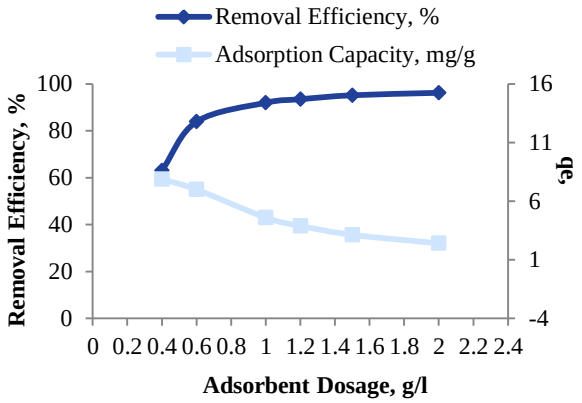

Figure 8. Comparison of the effect of adsorbent dose changes on the removal efficiency of chromium using MCh/Fe-Zr nanoparticles
(density Cr^{6+} = 5ppm, speed = 300 rmp, time = 30 minutes, temperature = 25 °C, and pH = 4)

Examination of the Effect of the Initial Concentration of Metal Ions on Adsorption Rate

The removal percentage of Cr^{6+} and Cu^{2+} using MCh/Fe-Zr nanoparticles under the effect of the initial concentration of metal ions ranging between 2 and 10 g/l was investigated for Cr^{6+} in pH of 4 and the adsorbent amount of 2 g/l, and for Cu^{2+} in pH of 9 and the adsorbent amount of 0.4 g/l. Figures 9 and 10 indicate the effect of the initial concentration of metal ions on removal percentage and adsorption capacity. As the initial concentration of the metal ions increased, the removal percentage dropped.
An increase in the initial concentration of metal ions causes gradient driving force of concentration to rise and enhance adsorption capacity. At low concentrations, all metal ions react with adsorption sites in the adsorbent; however, there are still free adsorption sites on the surface of the adsorbent. In high concentrations of metal ions, each active adsorption sites is surrounded by more metal ions; therefore, adsorption capacity increases as more adsorption sites are occupied. In higher concentrations, adsorption capacity is almost fixed, which is due to the saturation of adsorption sites.[18,19] Swayampakula et al. studied the removal of copper ions and reported similar results.[24]They found that as the concentration of ionic solution increased

from 50 to 200 mg/l, adsorption capacity also increase.[24] They reported a maximum adsorption capacity of 196 mg using 5 g/l adsorbent and time of 4 hours.[24]Zareie et al. studied the removal of copper using nano-chitosan and reported an increase in concentration from 10 to 100 mg/l, a decrease in removal efficiency, and 26.88 mg/g maximum removal capacity in concentration of 100 mg/l.[25]

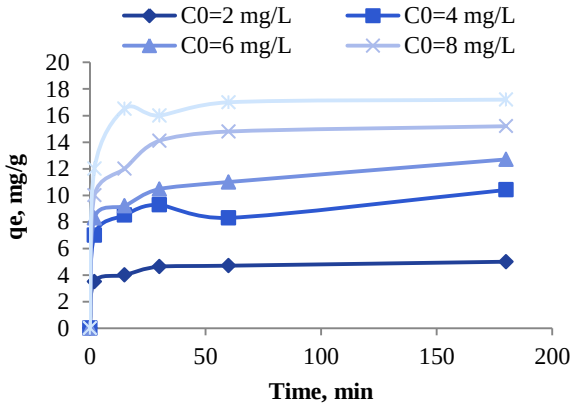

Figure 9. The effect of time and initial concentration on adsorption of copper on MCh/Fe-Zr nanoparticles in temperature 25 °C, adsorption dose of 0.4 g/l, speed of 300 rmp, time of 300 minutes, and pH of 9

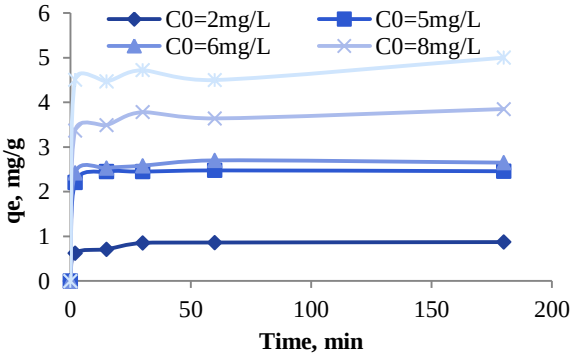

Figure 10. Comparison of the effect of time and initial concentration on adsorption of chromium on MCh/Fe-Zr nanoparticles in temperature of 25 °C, adsorption dose of 2 g/l, speed 300 of rmp, time of 60 minutes, and pH of 9

Adsorption Isotherm Models

As seen in figure 11 and table 1, the empirical data are in agreement with both equations. Therefore, it can be concluded that the surface of the adsorbent is homogeneous, and superficial adsorption occurs both physically and chemically.

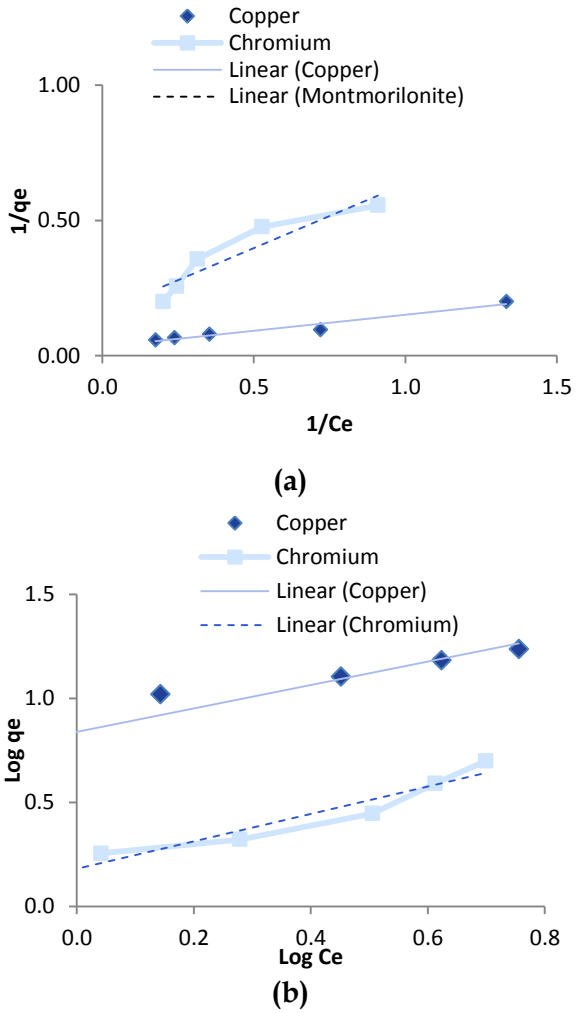

Figure 11. Langmuir (a) and Freundlich (b) adsorption isotherms of Cr and Cu ions using MCh/Fe-Zr nanoparticles in adsorbent dosage of 0.4 g/l for copper and 2 g/l for chromium, initial concentration of metal ions of 2-10 mg/l, temperature of 25 °C, Cu solution pH of 9 and Cr solution pH of 4

Table 1. Resulting parameters from adsorption isotherm models

Metal ion	Freundlich			Langmuir		
	K_f(l/mg)	n	R^2	K_l (l/mg)	q_{max}(mg/g)	R^2
Cr^{6+}	1.52	1.52	0.91	3.99	6.19	0.87
Cu^{2+}	6.91	1.78	0.91	0.28	30.07	0.95

Conclusion

In this study, the adsorption of Cu (II) and Cr (VI) ions onto MCh/Fe-Zr nanoparticles was studied. The effects of adsorption parameters, such as adsorbent dosage, pH, metal ion concentration, and adsorption isotherm, were investigated. The results indicate that due to having large superficial area and possessing amine and carboxyl functional groups, chitosan nanoparticles are effectively able to remove the metal ions of chromium and copper. Factors like pH, adsorption amount, and initial concentration of metal ions influence the maximum adsorption capacity. The maximum adsorption capacity of Cr^{6+} was obtained in pH of 4, adsorbent amount of 2 g/l, and concentration of 10 mg/g. The adsorption capacity of copper was the same in pH of 9, adsorbent amount of 0.4 g/l, and concentration of 10 mg/g.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

The authors would like to thank Shahid Sadoughi University of Medical Sciences for the financial and technical support of this study.

References

1. Naghizadeh A. Comparison between activated carbon and multiwall carbon nanotubes in the removal of cadmium(II) and chromium(VI) from water solutions. J Water Supply Res T 2015; 64(1): 64-73.
2. Dan Y, Hasfalina CM, Maryam RZ, Luqman CA, Rashid M. 2nd international conference on chemistry and chemical process (ICCCP 2012) May 5-6, 2012adsorption of copper (II) from aqueous medium in fixed-bed column by kenaffibres. APCBEE Procedia 2012; 3: 255-63.
3. Institute of Standards & Industrial Research of Iran. Water features [Online]. [cited 2013]; Available from: URL: http://khash.zaums.ac.ir/uploads/1053_27547.pdf
4. Tirtom VN, Dincer A, Becerik S, Aydemir T, Celik A. Comparative adsorption of Ni(II) and Cd(II) ions on epichlorohydrincrosslinked chitosan-clay composite beads in aqueous solution. Chemical Engineering Journal 2012; 197: 379-86.
5. Wan MW, Kan CC, Rogel BD, Dalida ML. Adsorption of copper (II) and lead (II) ions from aqueous solution on chitosan-coated sand. CarbohydrPolym 2010; 80(3): 891-9.
6. Ngah WSW, Fatinathan S. Adsorption of Cu(II) ions in aqueous solution using chitosan beads, chitosan-GLA beads and chitosan alginate beads. ChemEng J 2008; 143(1-3): 62-72.
7. Sun X, Peng B, Ji Y, Chen J, Li D. Chitosan(chitin)/cellulose composite biosorbents prepared using ionic liquid for heavy metal ions adsorption. AIChE Journal 2009; 55(8): 2062-9.
8. Dragan ES, Dinu MV, Timpu D. Preparation and characterization of novel composites based on chitosan and clinoptilolite with enhanced adsorption properties for Cu2+. BioresourTechnol 2010; 101(2): 812-7.
9. Dinu MV, Dragan ES. Evaluation of Cu2+, Co2+ and Ni2+ ions removal from aqueous solution using a novel chitosan/clinoptilolite composite: Kinetics and isotherms. ChemEng J 2010; 160(1): 157-63.
10. Huang G, Zhang H, Shi JX, Langrish TA. Adsorption of Chromium(VI) from Aqueous Solutions Using Cross-Linked Magnetic Chitosan Beads. IndEngChem Res 2009; 48(5): 2646-51.
11. Zhu HY, Fu YQ, Jiang R, Yao J, Xiao L, Zeng GM. Novel magnetic chitosan/poly(vinyl alcohol) hydrogel beads: Preparation, characterization and application for adsorption of dye from aqueous solution. BioresourTechnol 2012; 105: 24-30.
12. Fan L, Zhang Y, Li X, Luo C, Lu F, Qiu H. Removal of alizarin red from water environment using magnetic chitosan with Alizarin Red as imprinted molecules. Colloids Surf B Biointerfaces 2012; 91: 250-7.
13. Wang J, Xu W, Chen L, Huang X, Liu J. Preparation and evaluation of magnetic nanoparticles impregnated chitosan beads for arsenic removal from water. ChemEng J 2014; 251: 25-34.
14. Chen AH, Liu SC, Chen CY, Chen CY. Comparative adsorption of Cu(II), Zn(II), and Pb(II) ions in aqueous solution on the crosslinked chitosan with epichlorohydrin. J Hazard Mater 2008; 154(1-3): 184-91.
15. Ekhlasi L, Younesi H, Mehraban Z, Bahramifar N. Synthesis and application of chitosan nanoparticles for removal of lead ions from aqueous solutions. Water and Wastewater 2013; 24(1): 10-8.
16. Sreejalekshmi KG, Krishnan KA, Anirudhan TS. Adsorption of Pb(II) and Pb(II)-citric acid on sawdust activated carbon: Kinetic and equilibrium isotherm studies. J Hazard Mater 2009; 161(2-3): 1506-13.

17. Chu KH. Removal of copper from aqueous solution by chitosan in prawn shell: adsorption equilibrium and kinetics. J Hazard Mater 2002; 90(1): 77-95.
18. VasconcelosHelder L, Camargo TP, Gonsalves NS, Neves A, Laranjeira MCM, Favere VT. Chitosan crosslinked with a metal complexing agent: Synthesis, characterization and copper(II) ions adsorption. React FunctPolym 2008; 68(2): 572-9.
19. Naghizadeh A, Nabizadeh R. Removal of reactive blue 29 dye by adsorption on modified chitosan in the presence of hydrogen peroxide. Environ Prot Eng 2106; 42 (1): 149-168.
20. Perez-Fonseca A, Gomez C, Davila H, Gonzalez-Nunez R. Chitosan Supported onto Agave Fiber-Postconsumer HDPE Composites for Cr(VI) Adsorption. Ind Eng Chem Res 2012; 51(17): 5939-46.
21. Liu T, Yang X, Wang ZL, Yan X. Enhanced chitosan beads-supported Fe(0)-nanoparticles for removal of heavy metals from electroplating wastewater in permeable reactive barriers. Water Res 2013; 47(17): 6691-700.
22. Yu Z, Zhang X, Huang Y. Magnetic Chitosan-Iron(III) Hydrogel as a fast and reusable adsorbent for chromium(VI) removal. Ind Eng Chem Res 2013; 52(34): 11956-66.
23. Keng PS, Lee SL, Ha ST, Hung YT, Ong ST. Removal of hazardous heavy metals from aqueous environment by low-cost adsorption materials. Environ ChemLett 2014; 12(1): 15-25.
24. Swayampakula K, Boddu VM, Nadavala SK, Abburi K. Competitive adsorption of Cu (II), Co (II) and Ni (II) from their binary and tertiary aqueous solutions using chitosan-coated perlite beads as biosorbent. J Hazard Mater 2009; 170(2-3): 680-9.
25. Zareie C, Najafpour G, Sharifzadeh M. Preparation of Nanochitosan as an Effective Sorbent for the Removal of Copper Ions from Aqueous Solutions. Int J Eng 2013; 26(8): 829.

Journal of Advances in Environmental Health Research

Exploratory analysis of $PM_{2.5}$ variation trend of Tehran, Iran, in various time series and its relation with cardiovascular mortality rate using R software

Payman Kaseb[1], Ramin Nabizadeh[2], Kazem Nadafi[2], Kamyar Yaghmaeian[3]

1 Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2 Center for Air Pollution Research (CAPR), Institute for Environmental Research (IER) AND Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
3 Center for Water Quality Research, Institute of Environmental Research (IER) AND Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Original Article

Abstract

Among the numerous air pollutants, the strongest proof for adverse health effects has been reported for particulate matter (PM). The aim of this study was the exploration of short-term associations of air pollution with mortalities due to cardiovascular diseases (CVD) in Tehran, Iran, based on hospital and census data from 2007 to 2013. This descriptive and analytical research was conducted in 2015. Daily and hourly pollutant concentration was obtained from Tehran Metropolitan Municipality. Mortality rate records were obtained from the Ministry of Health, Central Municipal Cemetery, and Forensic Organization. In this study, data were analyzed using R software. Zoo, Time series, Stats, ts model, and Splines software packages were installed on R platform in order to outline the trend of different variables. The results showed that accidental mortality did not follow a particular trend and non-accidental mortality followed a descending or ascending trend. However, mortality pattern showed a decreasing trend from 2011 until the end of 2012. From the beginning of 2013, mortality pattern showed increasing trend. Moreover, the direct correlation of mortality rate and $PM_{2.5}$ concentration can be observed in a yearly and weekly time scale. Proof of a determined effect of airborne particles on mortality was found with $PM_{2.5}$. In addition, it was found that mortality rate shows a strong seasonal pattern, with a peak in winter and a minimum in fall. The peak the mortality rate in winter is most probably due to the spread of infectious diseases such as influenza as well as temperature-related phenomena in cold weather areas.

KEYWORDS: Air Pollution, Mortality, $PM_{2.5}$, R software

Date of submission: 15 May 2015, ***Date of acceptance:*** 24 Jul 2015

Citation: Kaseb P, Nabizadeh R, Nadafi K, Yaghmaeian K. **Exploratory analysis of PM2.5 variation trend of Tehran, Iran, in various time series and its relation with cardiovascular mortality rate using R software.** J Adv Environ Health Res 2015; 3(4): 276-83.

Introduction

During the previous 50 years, air pollution and its impact on individuals' health and the environment have been a global concern. The negative health effects of air pollution consist of a broad range of acute and chronic health effects causing increased hospital admissions,[1] increased emergency room visits,[2] and, most importantly, increased mortality.[3] The World Health Organization (WHO) estimated that ambient (outdoor) air pollution in both cities and rural areas caused 3.7 million premature deaths worldwide in 2012.[4] Epidemiological studies have revealed short-term and long-term associations between the levels of ambient air pollutants, and respiratory and cardiovascular mortalities in different parts of the world.[3]

Corresponding Author:
Ramin Nabizadeh
Email: rnabizadeh@tums.ac.ir

Previous studies regarding air pollution have focused mostly on nitrogen dioxide (NO_2), particulate matter (PM) with an aerodynamic diameter of less than 10 mm (PM10), sulfur dioxide (SO_2), or ozone (O_3).[4] Among the numerous air pollutants, the strongest proof for adverse health effects has been reported for PM.[5] Several studies have shown that short-term exposure to PM was associated with increased risk of mortality and morbidity.[6,7] The sources and constituents of the PM mixture are well-known to vary throughout the year. Therefore, besides the different exposure patterns of the population in different seasons, it is believed that the short-term relations between particulate air pollution and daily mortality can also change from season to season.[8] PM with an aerodynamic diameter of less than 2.5 mm ($PM_{2.5}$) has become the focus in recent research's due to its small size and capability to penetrate deep into the respiratory tract.[9] Inflammation, endothelial dysfunction, and autonomic nervous system injuries in rats due to $PM_{2.5}$ exposure have been reported in a recent study.[10] A committee of the American Heart Association in a review in 2004 expressed concern of a causal association between PM and harmful cardiovascular effects.[9] However, other adverse effects of $PM_{2.5}$ exposure have been reported in recent studies. For example, Santos et al. stated that exposure to PM could increase the risk of ventricular tachycardia for aging people with coronary artery disease (CAD).[11] Nevertheless, Xu et al. reported that cardiac autonomic function of elderly patients with heart disease can result from ambient $PM_{2.5}$ exposure, and subjects with hypertension seemed to be more susceptible to the autonomic dysfunction caused by $PM_{2.5}$.[12] Tehran, as the most overcrowded city in Iran, has experienced serious air quality problems in the recent years. Today, traffic, industrial processes, domestic heating, long-range transportation of pollutants and motor vehicle are the most significant emission sources in Tehran. With regard to the aforementioned pollution source, high levels of air pollution-related diseases are expected in Tehran. In Iran a number of studies have been conducted on air pollution related diseases.[13,14] Conversely, the association between $PM_{2.5}$ and cardiovascular morbidities has not been surveyed locally and in Iran. This study was conducted with the aim to explore short-term associations of air pollution with morbidities and mortalities caused by cardiovascular diseases (CVDs) in Tehran based on hospital and census data from 2007 to 2013.

Materials and Methods

Tehran (capital of Iran) is the largest city and urban area of Iran, the 2nd-largest city in Western Asia with an area of 686.3 km^2. Tehran County borders Shemiranat County to the north, Damavand County to the east, Eslamshahr, Pakdasht, and Rey counties to the south, and Karaj and Shahriar counties to the west. The city of Tehran has 22 municipal districts, each with its own administrative center. Tehran features a semi-arid climate with continental climate characteristics and a mediterranean climate precipitation pattern. Average temperatures in Tehran are between 35 and 40 °C. Most of the light annual precipitation occurs from late autumn until mid-spring.[15] Meteorological factors have significant effect on air pollution. Wind carries air pollutants away from their source, causing them to disperse. Generally, in higher wind speed, more pollutants are distributed and their concentrations are lowered. However, high wind speed can also transport dust from long range distances.[16]

This descriptive and analytical research was conducted in 2015. Daily and hourly pollutant concentration was obtained from the 34 air quality stations of Tehran Metropolitan Municipality. The air quality indicator in our study was PM with aerodynamic diameter of less than 2.5 µm ($PM_{2.5}$). Mortality rate records of 2007-2013 were obtained from the Ministry of Health, Central Municipal Cemetery, and Forensic

Organization. Meteorological and demographic information of 22 districts of Tehran were obtained from the Meteorological office in Tehran. Deaths from all CVDs were considered.

The locations of air quality monitoring stations are shown in figure 1. A lack of uniformity was observed in the distribution of contaminants in different areas of Tehran, and thus, there was a possibility of data misinterpretation. Therefore, trimmed mean of pollutant concentration in the range of 10% was used instead of daily means of the concentrations calculated from the hourly data of pollutant. Trimmed mean is a reliable averaging method in which the highest and lowest reported data is discarded, and then, the average is taken from the remaining uniform data.[17]

After editing of incorrect and irrelevant data, all cases of mortality were classified based on age, sex, place of residence, and cause of death. In order to remove confounding factors and achieve correct results, all cases of death of 65 years of age or younger were selected. Given that the main objective of this research was the assessment of the relation of air pollution with mortality rate, it was necessary to discard deaths caused by accidents, cancers, and chronic CVD.

To evaluate the impact of air pollution on human health, epidemiological studies practice statistical approaches which are suitable tools for interpreting data. Time series studies are often used to determine statistical relations of ambient concentrations of pollutants and other environmental factors with mortality. The most favored statistical method over the past two decades has been Poisson regression with air pollution variables included as linear predictors and monitoring for time-varying confounders which are feasibly related both to mortality and air pollution.[18]

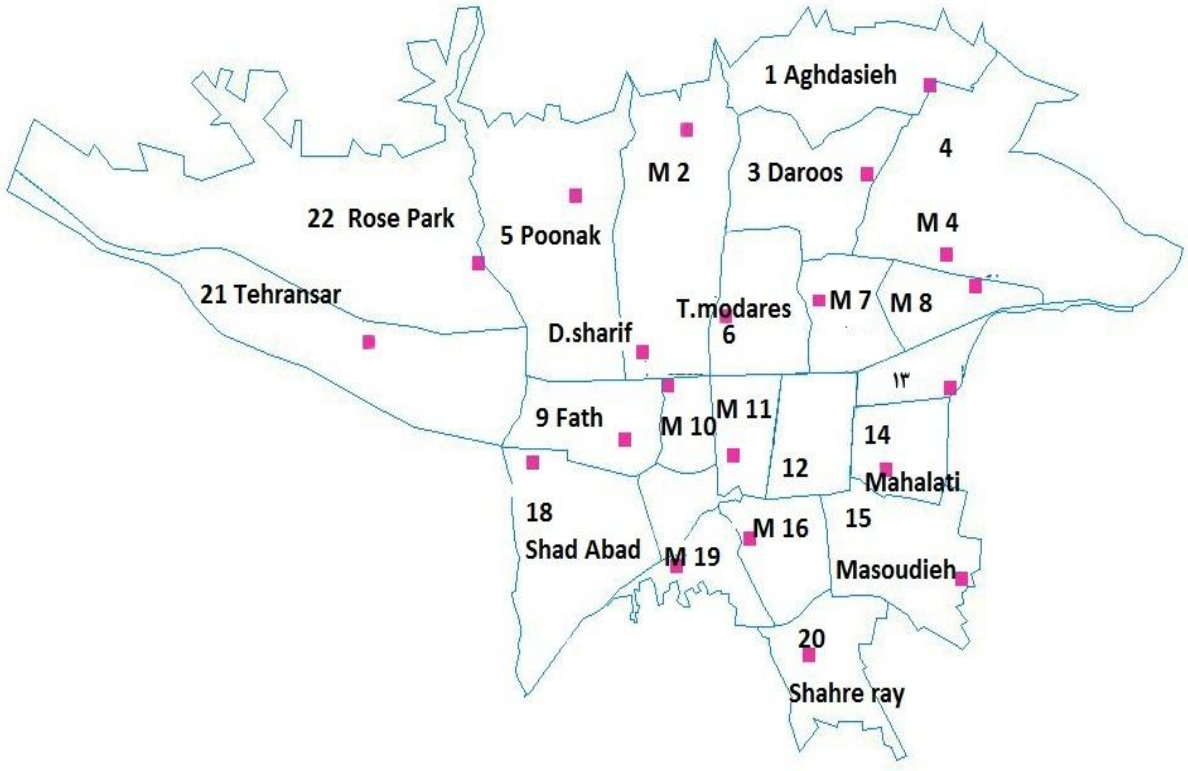

Figure1. Map of the study area including air quality monitoring stations

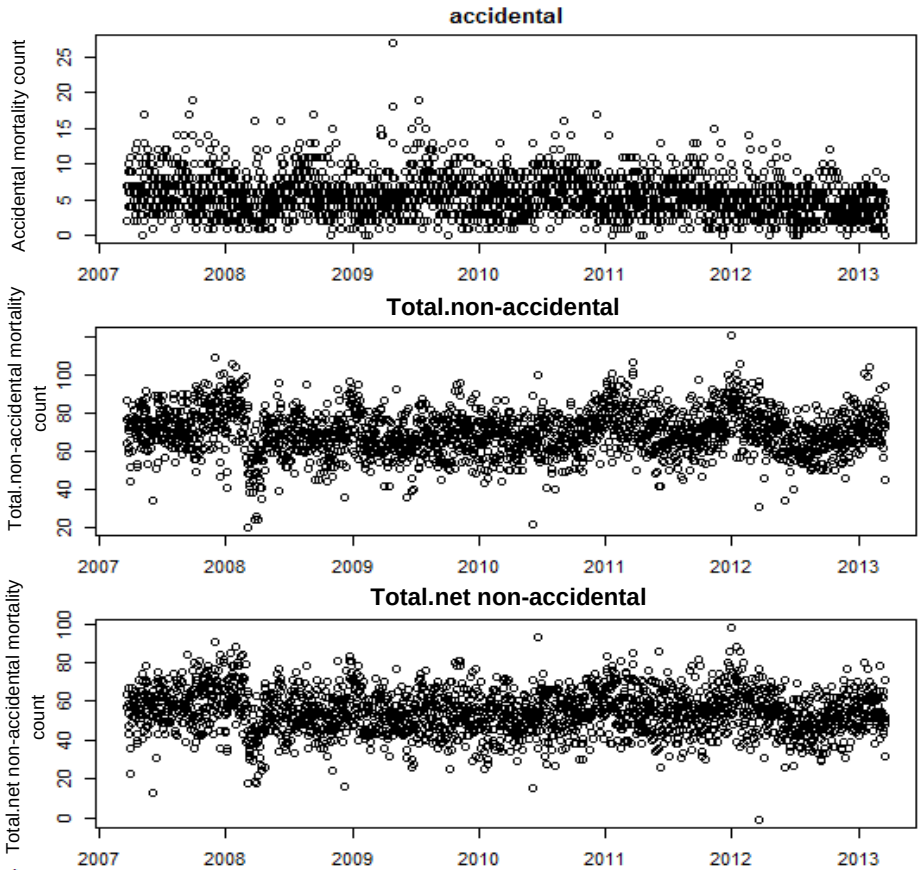

Figure 2. Daily mortality data on cases aged 65 years or younger in Tehran during 2007-2013

In this study, data were analyzed using R software (version 3.2, R Core Team). Zoo, time series, stats, ts model, and Splines software packages were installed on R platform in order to outline the trends of different variables. Moreover, the plot of the autocorrelation function (ACF) of the residuals was examined. To estimate linear trends of short-term effects of $PM_{2.5}$ on mortality, statistical tools developed by Peng et al.[19] were adapted to estimate time-varying relative rates.[20]

Results and Discussion

The mortality data for cases aged 65 or younger is presented in figure 2. As shown in figure 2, accidental mortality did not follow a particular trend. By excluding accidental mortality, non-accidental mortality followed a descending or ascending trend. In addition, figure 2 shows that net non-accidental mortality has the same pattern as non-accidental mortality.

Figure 3 shows $PM_{2.5}$ time series decomposition from 2007 to 2013. It appears that daily concentration of $PM_{2.5}$ has had a descending trend from 2011 to 2012. At the end of 2012, daily concentration of $PM_{2.5}$ showed an increasing trend. It can also be observed in figure 3 that the evident seasonal pattern in $PM_{2.5}$ concentration showed a peak in winter.

Environmental regulations proposed to protect human health are based on a foundation of scientific proof that arises from toxicological, clinical, and epidemiologic research. Their purpose is to reduce exposure to agents that have been found to harmfully affect health, and their application is thought to lead to a reduction in the burden of health effects caused by the exposure.[21]

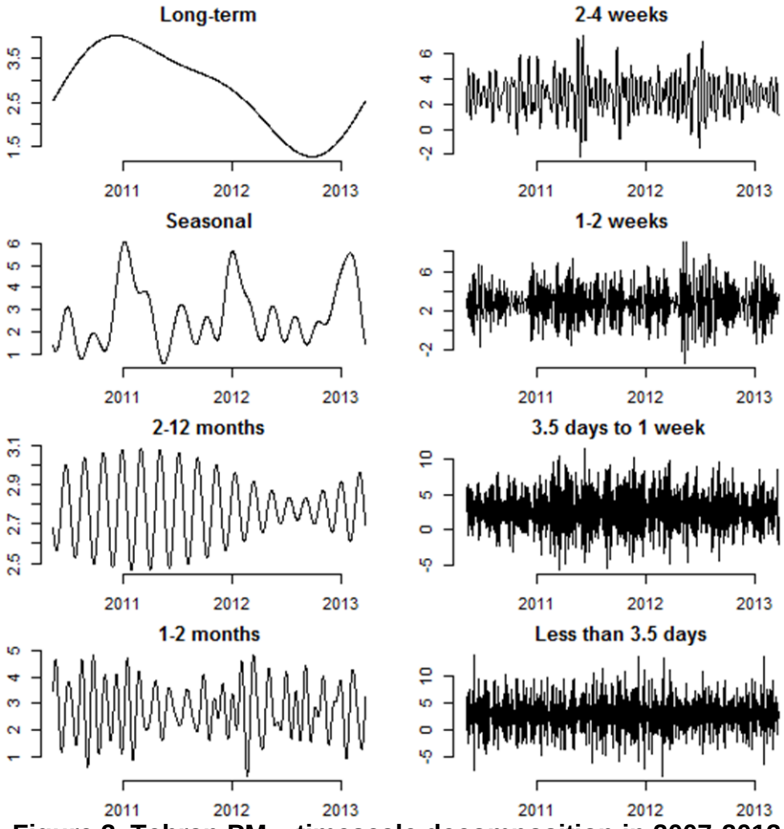

Figure 3. Tehran $PM_{2.5}$ timescale decomposition in 2007-2013

This paper presents evidence on the short-term effects of $PM_{2.5}$ on mortality during 2007-2013, when several air quality regulations were implemented. Specifically, changes in the risk of exposure to airborne particles were assessed over a period of substantial alterations in the sources and decline in ambient concentrations of airborne particles. For this purpose, almost all available data on $PM_{2.5}$ and mortality were used. As a result, proof of a determined effect of airborne particles on mortality was found with $PM_{2.5}$. In a study conducted by Shahi et al., effects of air pollution on cardiovascular and respiratory causes of emergency admission were evaluated.[22] The results of their study showed that carbon monoxide (CO) level was an independent risk factor of CVD while the increased level of $PM_{2.5}$ and O_3 led to increased rate of admissions to the emergency department due to respiratory causes.[22]

Figure 4 shows time series mortality rates among individuals of less than 65 years of age. As seen in figure 4, the mortality pattern shows a decreasing trend from 2011 until the end of 2012. From the beginning of 2013, mortality pattern shows an increasing trend.

Mortality rate showed a strong seasonal pattern with a peak in winter and a minimum in fall. However, a lower amount of $PM_{2.5}$ was observed in winter. Thus, the peak in winter mortality is most probably due to the spread of infectious diseases, such as influenza, as well as temperature-related phenomena in cold weather areas. Higher amount of $PM_{2.5}$ during fall can be related to car traffic since the schools open in the fall. According to our study, the risk of mortality due to air pollution can occur up to 50 days (50 days lag) after exposures especially lag 0. Goudarzi et al. studied the relationship between air pollution exposure and chronic obstructive pulmonary disease (COPD) in Ahvaz, Iran, in 2012.[23] The results of their study showed that the annual average PM_{10} concentration in 2012 was 727 $\mu g/m^3$. According to their results, a strong

correlation was observed between hospital visits due to COPD and PM_{10} emission in Ahvaz. In addition, they found that approximately 6.2% of hospital admissions due to COPD occurred when PM_{10} concentration was higher than 30 µg/m³.

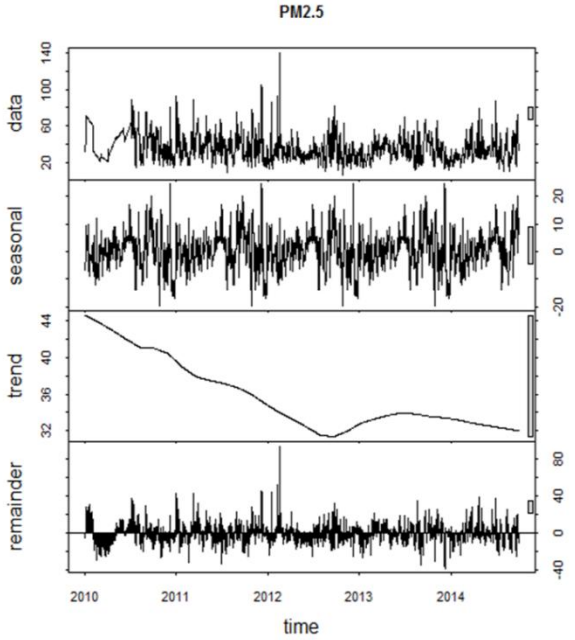

Figure 4. Tehran mortality timescale decomposition among individuals of less than 65 years of age in 2007-2013

Figure 5(a) shows a correlogram for the mortality data on Tehran. The correlogram can be computed in R using the ACF function in the stats package. The result shows a peak in lag 0. In other words, the plot shows the quick effect of $PM_{2.5}$ on mortality rate in the first day.

Nevertheless, a mild effect was observed in lag 5, 8, 15, and 21. Figure 4(b) shows the correlogram of the residuals after removing some of the seasonality of mortality data. The results showed that the effect of $PM_{2.5}$ on mortality was observed specifically in lag 0.

Cardiovascular mortality was associated especially with daily mean $PM_{2.5}$. Furthermore, $PM_{2.5}$ was not significantly associated with total non-accidental mortality. Based on ACF analysis, the results of the present study showed that $PM_{2.5}$ impacted mortality rate. In other cases, we have not seen any evident effect. This suggests that other seasonal diseases have a significant effect on mortality. Capraz et al. studied the association between air pollution and mortality in Istanbul, Turkey.[3] Their findings showed that the risk of mortality due to air pollution can occur up to 10 days (10 days lag) after exposure. Moreover, their results showed that cardiovascular mortality was related to daily mean SO_2, followed by NO_2 and PM_{10}.[3]

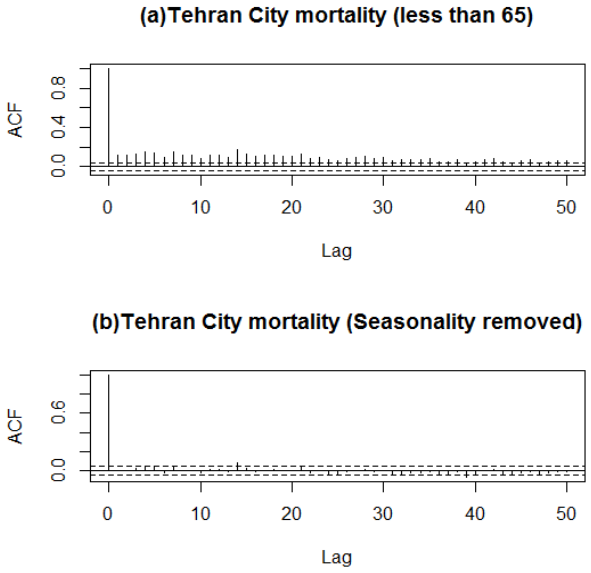

Figure 5. Autocorrelation functions for mortality data on Tehran for (a) raw data and (b) residuals after removing seasonality

Simultaneous changes in mortality rate and $PM_{2.5}$ concentration in annual, seasonal, and weekly time scale are shown in figure 6. As figure 6 shows, direct correlation between mortality rate and $PM_{2.5}$ concentration can be observed in the annual and weekly time scale. Valid correlation was not found in the seasonal time scale. In a study conducted by Lu et al., it was found that associations between air pollution and mortality were more distinct in the warm season than in the cool season.[24] They concluded that the increase in risk of mortality corresponded to an increase in current ambient concentrations of air pollutants. Qiu et al. studied the association between air pollution and

mortality in china.[25] They found that for a 10 µg/m³ increase in pollution concentration, a 1.6%-2.3% additional increase in mortality related to PM, NO_2, and SO_2 was observed among individuals aged 65 years or older compared with younger individuals.

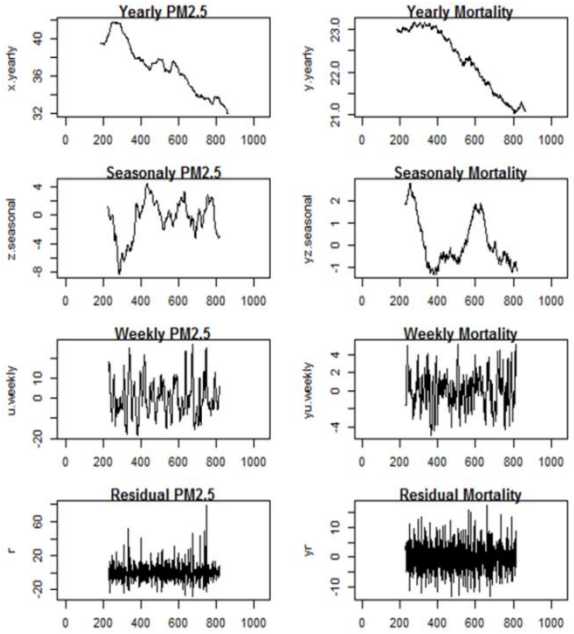

Figure 6. Timescale decomposition for $PM_{2.5}$ and mortality data in Tehran during 2007-2013

Conclusion

Our results showed that short-term exposure to outdoor air pollution ($PM_{2.5}$) was associated with CVD in Tehran between 2007 and 2013. High percentages of observed health effect (CVDs) were linked with high concentrations of $PM_{2.5}$. Therefore, application of methods to reduce $PM_{2.5}$ concentration plus suitable health and environmental monitoring are recommended. In the present study, it was also found that day-to-day variations in cardiovascular mortality are associated with ambient concentrations of $PM_{2.5}$. According to the results of this study, the risk of mortality due to air pollution can be observed up to 50 days (50 days lag) after the exposure.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

This study was funded by the Vice Chancellery for Research of Tehran University of Medical Sciences, Iran.

References

1. European Centre for Environment and Health. Quantification of the health effects of exposure to air pollution. Proceedings of the WHO Working Group; 2000 Nov 20-22; Bilthoven, Netherlands.
2. Buka I, Koranteng S, Osornio-Vargas AR. The effects of air pollution on the health of children. Paediatr Child Health 2006; 11(8): 513-6.
3. Capraz O, Efe B, Deniz A. Study on the association between air pollution and mortality in stanbul, 2007-2012. Atmos Pollut Res 2016; 7(1): 147-54.
4. Tam WWS, Wong TW, Wong AHS. Association between air pollution and daily mortality and hospital admission due to ischaemic heart diseases in Hong Kong. Atmos Environ 2015; 120: 360-8.
5. Pope CA 3rd, Dockery DW. Health effects of fine particulate air pollution: lines that connect. J Air Waste Manag Assoc 2006; 56(6): 709-42.
6. Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. Circulation 2010; 121(21): 2331-78.
7. Kloog I, Ridgway B, Koutrakis P, Coull BA, Schwartz JD. Long-and short-term exposure to PM2.5 and mortality: using novel exposure models. Epidemiology 2013; 24(4): 555-61.
8. Chen R, Peng RD, Meng X, Zhou Z, Chen B, Kan H. Seasonal variation in the acute effect of particulate air pollution on mortality in the China Air Pollution and Health Effects Study (CAPES). Sci Total Environ 2013; 450-451: 259-65.
9. Cao J, Xu H, Xu Q, Chen B, Kan H. Fine particulate matter constituents and cardiopulmonary mortality in a heavily polluted Chinese city. Environ Health Perspect 2012; 120(3): 373-8.
10. Wang G, Zhen L, Lu P, Jiang R, Song W. Effects of ozone and fine particulate matter (PM2.5) on rat cardiac autonomic nervous system and systemic inflammation. Wei Sheng Yan Jiu 2013; 42(4): 554-60.
11. Santos UP, Terra-Filho M, Lin CA, Pereira LA, Vieira TC, Saldiva PH, et al. Cardiac arrhythmia emergency room visits and environmental air pollution in Sao Paulo, Brazil. J Epidemiol Community Health 2008; 62(3): 267-72.
12. Xu MM, Jia YP, Li GX, Liu LQ, Mo YZ, Jin XB, et al. Relationship between ambient fine particles and ventricular repolarization changes and heart

rate variability of elderly people with heart disease in Beijing, China. Biomed Environ Sci 2013; 26(8): 629-37.
13. Gholizadeh MH, Farajzadeh M, Darand M. the correlation between air pollution and human mortality in Tehran. Hakim Res J 2009; 12(2): 65-71. [In Persian].
14. Mousavi F, Jahed A, Rajab A, Nikuo Sokhantabar AK, Kashi G, Tabatabaee R. Survey of air pollution effect on variation of glycosylated hemoglobin a1c (hba1c) level in diabetic patients in Tehran. Iran J Health Environ 2013; 6(1): 123-32. [In Persian].
15. Tehran Municipality. Atlas of Tehran [Online]. [cited 2016]; Available from: URL: http://atlas.tehran.ir/Default.aspx?tabid=40
16. Ramos DP, Quijano S, Orrego D, Becerra MA. Dependence analysis using multiple linear regresion and principal component regression in air pollutants and meteorological parameters: case study. QUID: Investigacion, Ciencia y Tecnologia 2014; (23): 13-20.
17. Hellerstein JM. Quantitative data cleaning for large databases [Online]. [cited 2008 Feb 28]; Available from: URL: http://db.cs.berkeley.edu/jmh/papers/cleaning-unece.pdf
18. Anderson HR. Air pollution and mortality: A history. Atmos Environ 2009; 43(1): 142-52.
19. Peng RD, Dominici F, Pastor-Barriuso R, Zeger SL, Samet JM. Seasonal analyses of air pollution and mortality in 100 US cities. Am J Epidemiol 2005; 161(6): 585-94.
20. Chiogna M, Gaetan C. Dynamic generalized linear models with application to environmental epidemiology. J R Stat Soc Ser C Appl Stat 2002; 51(4): 453-68.
21. Dominici F, Peng RD, Zeger SL, White RH, Samet JM. Particulate air pollution and mortality in the United States: did the risks change from 1987 to 2000? Am J Epidemiol 2007; 166(8): 880-8.
22. Shahi AM, Omraninava A, Goli M, Soheilarezoomand HR, Mirzaei N. The Effects of Air Pollution on Cardiovascular and Respiratory Causes of Emergency Admission. Emerg (Tehran) 2014; 2(3): 107-14.
23. Goudarzi G, Geravandi S, Mohammadi MJ, Salmanzadeh S, Vosoughi M, Sahebalzamani M. The relationship between air pollution exposure and chronic obstructive pulmonary disease in Ahvaz, Iran. Chron Dis J 2015; 3(1): 14-20.
24. Lu F, Zhou L, Xu Y, Zheng T, Guo Y, Wellenius GA, et al. Short-term effects of air pollution on daily mortality and years of life lost in Nanjing, China. Sci Total Environ 2015; 536: 123-9.
25. Qiu H, Tian L, Ho KF, Pun VC, Wang X, Yu IT. Air pollution and mortality: effect modification by personal characteristics and specific cause of death in a case-only study. Environ Pollut 2015; 199: 192-7.