Original Article

Microbiological and Chemical Findings of Water Used for Various Industrial Processes in Babol Car Factory, Iran, in 2013; A Case Study

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Abstract

Background and purpose: According to the reported problems in area of the inappropriateness of water quality which used for washing and staining of car apparatuses in a car factory, this research was carried out for identification of physical, chemical, and microbiological characteristics of the consumed water in Babol car factory, Iran.

Materials and Methods: Physical and chemical parameters of water such as total solids, total dissolved solids (TDS), turbidity, pH, electrical conductivity, total alkalinity, total hardness (TH), cations (Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}), and anions ($SO4^{2-}$, Cl^-, $NO3^-$) were analyzed based on standard methods for examination of water and wastewater. The samples were taken from five units and were precipitated and plated on Sabouraud dextrose agar supplemented with chloramphenicol and blood agar and eosin methylene blue media.

Results: TDS, turbidity, pH, TH and ammonium ion were 402 mg/L, 10 NTU, 7.8, 208 mg/L and 0.04 mg/L, respectively. Ten genera of fungal colonies were isolated from these units, which from them yeast; Penicillium and Cladosporium were the most prevalent. Five genera of bacteria were isolated from these samples. Entrobacteriaceae, Pseudomonas, and Bacillus were the most prevalent bacteria in water. Water quality in the activation and fixation units were the most contaminated with fungi and bacteria, respectively. Some of these units were without fungal and bacterial contaminations.

Conclusion: The fungal and bacterial contaminations can be changed the quality of consumed water in the different processes such as color and turbidity. Thus, we need to use some water treatment processes such as chemical disinfection or microfiltration for removal of microorganisms.

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Key words: Rainwater, Industrial water, Fungi, Bacteria, Car factory

1. Introduction

Air has a lot of pathogens and saprophyte microorganisms such as bacteria and fungi (1,2). Roof-harvested rain water has received significant attention as a potential alternative source of water (3,4). Many countries such as Australia, Denmark, Germany, India, and New Zealand are increasing studies around use of rainwater for some purpose (5). Many compounds such as organic and inorganic pollutants have the potential of entrance in rainwater. The main components of these contaminants are divided into five categories, which are summarized in figure 1 (6).
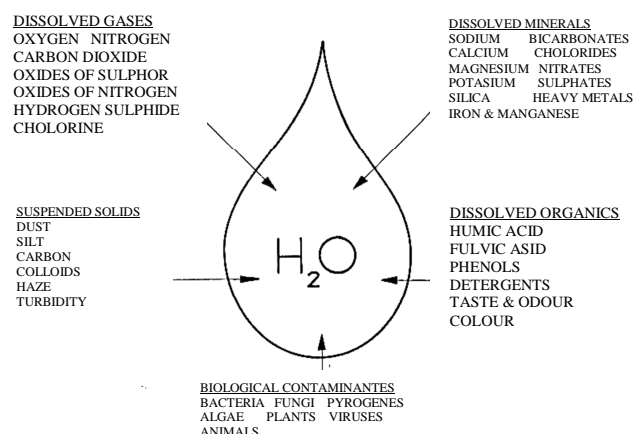

DISSOLVED GASES
OXYGEN NITROGEN
CARBON DIOXIDE
OXIDES OF SULPHOR
OXIDES OF NITROGEN
HYDROGEN SULPHIDE
CHOLORINE

DISSOLVED MINERALS
SODIUM   BICARBONATES
CALCIUM   CHOLORIDES
MAGNESIUM NITRATES
POTASIUM   SULPHATES
SILICA   HEAVY METALS
IRON & MANGANESE

SUSPENDED SOLIDS
DUST
SILT
CARBON
COLLOIDS
HAZE
TURBIDITY

DISSOLVED ORGANICS
HUMIC ACID
FULVIC ASID
PHENOLS
DETERGENTS
TASTE & ODOUR
COLOUR

BIOLOGICAL CONTAMINANTES
BACTERIA FUNGI PYROGENES
ALGAE   PLANTS   VIRUSES
ANIMALS

Figure 1. The different pathway entrances of contaminants into rainwater (6)

Water shortage is a serious problem today. According to UN projections, until 2025, nearly two-thirds of the world's population will face a shortage of drinking water (7). Iran is a country that suffers from water shortages. One of the most important resources of water used in each country is potable water consumption, agricultural and industrial using (8). Recent studies show that one of the most important strategies in countries facing water shortages is rainwater using (8,9).

Bioaerosols can the source of bacterial toxins and fungal allergens, thus the rain water has different types of organisms (1,10-12). They can enter in rainwater and cause waterborne diseases and many problems

in the different processes of industries, respectively (13,14). Considering to the types and quantities of the microorganisms in water, they cause some degradations in rainwater quality (15,16). Reservation of rainwater in units for different types of purpose can be affecting the amount of contaminations (17,18). The different types of filamentous and yeast fungi were isolated from air and rainwater, and these contaminations can raise some problems in physical characteristics of the industrial water such as turbidity and color (19,20). Metals especially iron have more important in the various industries Some studies showed that many mineral compounds such as Al are the cause of rain water pollution (6,8,9). Basic reasons of this matter are due to firmness, availability, cost-effective, and recyclability of these materials (21). One of the most important disadvantages of metals especially iron is rustiness and corrosion (22,23). Iron plates are rusted easily, during to storage, transportation, and formation in undesirable conditions. Metals insulation with some chemicals such as dyes and pigments is a useful method for conservation of metals against to corrosion (22). There are some problems in the area of metals painting in the different industries. Many of chemical compounds such as oil and grease and also some microorganisms (bacteria and fungi) cause many problems in the field of metals painting (23). Automobile manufacture industries are the most important industries in related to metals consumption (24). There are several methods to metals surface preparation for the proper painting in the car manufacturing industries (25,26). The most important methods for the metal surface preparation before the painting processes are: (1) Mechanical clean up method; In this method, in order to clean up surfaces of metals is used many inert washing solutions such as non-ionic surfactants by hand for grease and other agents removal, (2) Chemical clean up method - In this method, some

chemicals such as alkaline and acidic compounds is used to clean up oil and grease, bacteria and fungi on the surfaces of metal plates (22,23). According to many reports of some problems in water of metal plates painting in car factories, especially problem of turbidity and color of water consumed in the different units of Babol car factory, Iran. Therefore, the present study was carried out to determination of physical and chemical parameters, bacterial and fungal contaminations in the consumed water in the different units of a car factory in Babol.

2. Materials and Methods

The rainwater harvested is stored in cement tank. Then the water enters polyethylene tank. Third unit is mechanical cleaning basin that car apparatuses to grease removal clean with mechanical brush, water and detergent by hand. Next unit, these components are rinsed with water. Activation and phosphate units are the following processes for coloring the car components and color stabilization. In the next unit, the components are rinsed with water again. Finally, in the last unit, the components are sealed and rinsed with water (Figure 2).

The consuming water of Babol car factory was supplied from rainwater harvested. In order to determination of microbial population in the consumed water of in the investigated industry, five samples were taken from five different units contain cement tank, polyethylene tank, activation, phosphate, and sealing units. 10 ml of each sample collected from 30 cm under of water surface in sterile tubes, according to the water sampling standard (27). The samples transferred to Mycology and Microbiology Laboratories, Faculty of Medicine, Babol University of Medical Sciences. After removing of large particles, the samples were precipitated by centrifugation (2000 rpm for 10 min).

In this research, physical parameters of the consumed water such as total solids (TS), total dissolved solids (TDS) and turbidity, as well as chemical properties like pH, electrical conductivity, total alkalinity, total hardness (TH), cations (Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}), and anions ($SO4^{2-}$, Cl^-, $NO3^-$) were analyzed based on Standard Methods for examination of water and wastewater (27). In order to analyzing of microbial characteristics of the water samples, 1 ml of all precipitated samples was used for staining and culture. 20 µl of samples stained by methylene blue dye was examined microscopically for fungi and bacteria. 20 µl of them, plated on sabouraud dextrose agar supplemented with chloramphenicol for growth of fungi; and blood agar and eosin methylene blue for growth of bacteria. All plates incubated at room temperatures and 37°C for up to 48 h for bacteria and 2 weeks for fungi (18,19).
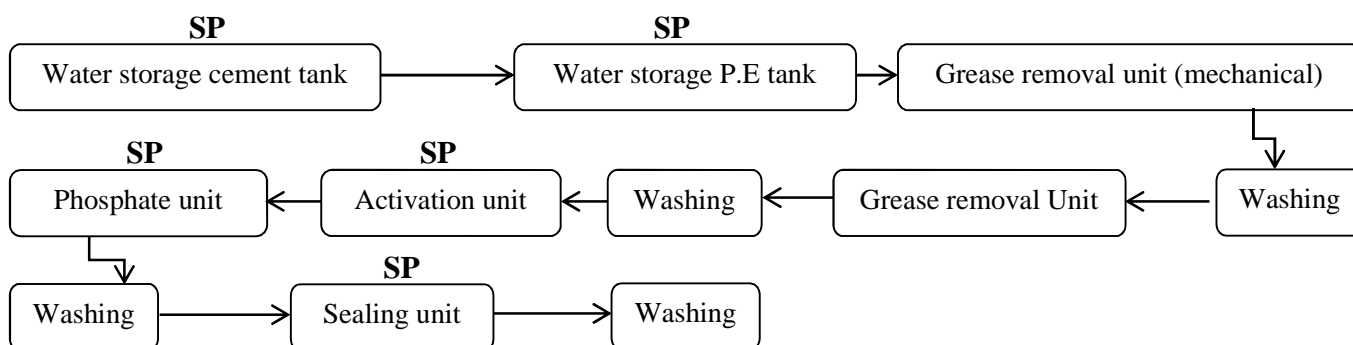

Figure 2. Flow diagram of industrial different processes and water sampling points (SP)

Fungi identified according to macroscopic and microscopic appearances; and also staining and slide culture method. Bacterial colonies identified according to macroscopic and microscopic characteristic appearances; and also staining and biochemical tests (20,28). The number of colonies calculated based amount of cultured on media and the number of microorganisms grew.

3. Results

Based on table 1, concentrations of the physical parameters such as TDS and turbidity were 402 mg/L and 10 NTU; chemical properties such as pH, TH, and ammonium ion were 7.8, 208 and 0.04 mg/L, respectively. In this study, all the parameters in the different stages in the factory are compatible with the national standard of Iran and WHO guideline. However, turbidity and ammonium ion concentrations in the consumed water (10 NTU and 0.04 mg/L) are more than the maximum allowable concentration of the national standard of Iran and WHO guideline (5 NTU and 0.02 mg/L).

In this study, 10 genera of fungal colonies and also five bacterial genera were isolated from units. Yeast (70.75%), Penicillium (16.51%), and Cladosporium (8.02%) were the most prevalent fungi (Table 2).

Table 1. Physicochemical characteristics of consuming raw water in various industrial processes in a car factory of Babol

Parameters	Babol car factory water	Iranian standard (29)	EPA standard (30)***	WHO guideline (31)
pH	7.8	6.5-8.5	6.5-8.5	6.5-8.5
Conductivity, µS/cm*	815	-	-	-
Turbidity, (NTU**)	10	5	5	5
TS, mg/L	522	1500	-	1500
TDS, mg/L	402	500	500	1000
Total alkalinity, mg/L	100	200	-	-
TH, mg/L	208	500	-	500
Ca^{2+}, mg/L	64	75	-	75
Mg^{2+}, mg/L	11.5	50	-	30
FE^{2+}, mg/L	0.1	0.3	0.3	0.3
Mn^{2+}, mg/L	0.03	0.05	0.05	0.05
Zn^{2+}, mg/L	0.1	5	5	5
$NH4^{+}$, mg/L	0.04	0.02	-	-
Cl^{-}, mg/L	105	200	250	250
$SO4^{2-}$, mg/L	125	200	250	250
$NO3^{-}$, mg/L (as $NO3^{-}$)	1.2	50	50	50
$NO2^{-}$, mg/L (as $NO2^{-}$)	0	3	3	3
Chlorine residual, mg/L	0.4	0.2-1	4	0.2- 0.8

*µS/cm: Micro siemens/cm; TS: Total solids; TDS: Total dissolved solids; TH: Total hardness; **NTU: Nephelometry turbidity unit; ***EPA: Environmental protection agency

**Table 2.** Fungi isolated from consuming water in various industrial processes of Babol car factory

Fungi	Location				
	Cement unit	Plastic unit	Activation unit	Fixation unit	Sealing unit
Acremonium sp.	0*	0	0	20	0
Aspergillus sp.	0	0	20	0	0
Bipolaris sp.	0	0	10	0	0
Penicillium sp.	40	0	130	0	180
Trichoderma sp.	0	0	20	0	0
Drecselera sp.	0	0	10	0	0
Fusarium sp.	0	0	50	0	0
Cladosporium sp.	70	0	30	70	0
Yeast	400	0	500	600	0
Mycelia sterile	10	0	10	10	30
Total	520	0	780	700	210

*CFU: Colony forming unit

Table 3. Bacteria isolated from consuming water in various industrial processes of Babol car factory

Bacteria	Place				
	Cement unit	Plastic unit	Activation unit	Fixation unit	Sealing unit
Staphylococcus(coagolase negative)	30*	0	0	0	0
Enterobacteriaceae	0	0	0	400	0
Bacillus sp.	0	0	150	0	0
Pseudomonas sp.	0	0	0	300	0
Diphteroid sp.	0	0	110	0	0
Total	30	0	260	700	0

*CFU: Colony forming unit
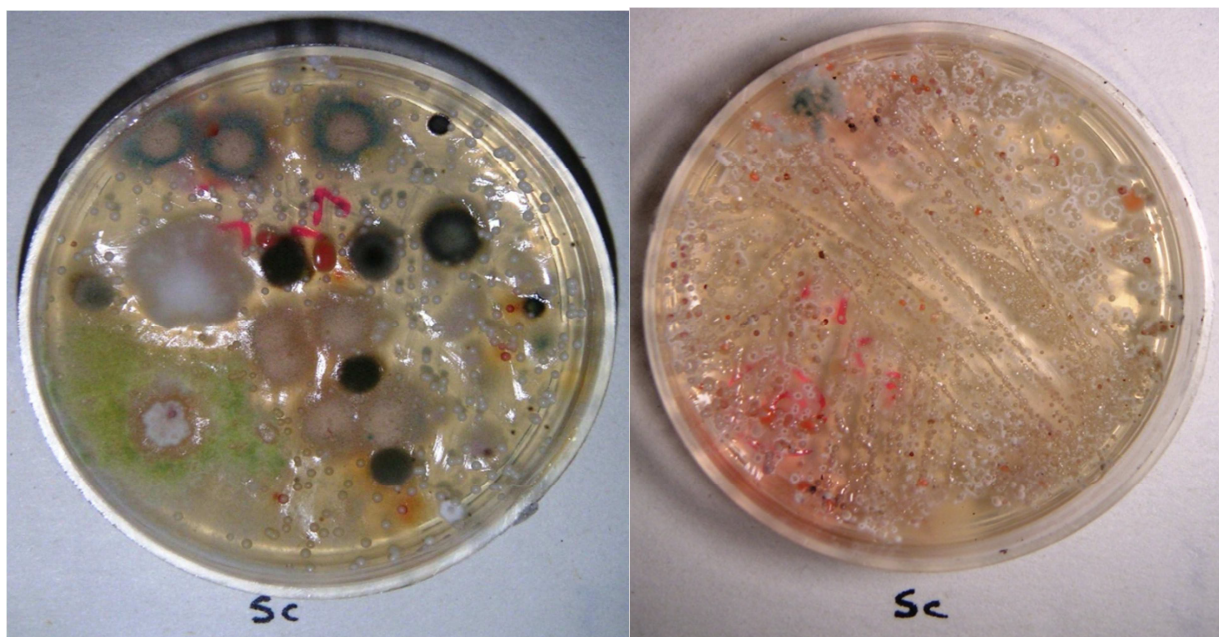
Figure 3. Fungal and bacterial colonies grown in water samples, (a) Activation unit, (b) fixation unit

Activation and fixation units were the most contaminated with fungi. Plastic unit was without any fungal contamination. Entrobacteriaceae (40.40%), Pseudomonas (30.30%), and Bacillus (15.15%) were grown more than other bacteria (Table 3).

Fixation and activation units were the most contaminated with bacteria (Figure 3), while plastic and sealing units were without any bacterial contamination. Plastic unit was without any both fungal and bacterial contamination.

4. Discussion

In this research, turbidity of water consumed was 10 NTU and is more than maximum allowable concentration of the national standard of Iran and WHO guideline (29,31).

Increasing of water turbidity cause magnifies microorganism's growth due to easy locating of bacteria and fungi on the suspended particles (32). Another factor of microorganism's growth in water is nitrogen compounds (32). In this study, ammonium ion concentration in the water was 0.04 mg/L that is more than maximum allowable concentration of the national standard of Iran. Ammonium ion can be resulted to microorganism's population increasing as nutrient in the water (32,33). The results showed that almost all of units were contaminated to bacteria and fungi; of course differ from each unit to another unit. Enterobacteriaceae and yeast in our study were 0.4×10^3 and 0.6×10^3 cells/ml, respectively. Totally, 80% and 60% of units

have been contaminated to fungi and bacteria, respectively. It seems the change of the mineral component in each unit affect the quantity and quality of microorganisms, for example fixating unit had the most microorganisms. Some metals and other materials are a suitable source for generation and induction of bacterial and fungal growth in incubated (13,34). Almost all of studies showed the rain harvested water is contaminated to bacteria, fungi and protozoa (8,10). These contaminations can be due to air and some small animals (11). In a study, from 27 rainwater samples, 17 (63%) were positive for Escherichia coli (8). In a research, Bacillus bacteria were isolated from 42% of samples (10). We need understand the risk and prediction of health hazards of bioaerosols, especially if it enters to water. According to the results of the present study, the fungal contamination can change the quality of rainwater such as color and turbidity. It is possible that this contamination was started from the first unit to next units and final products. Thus, we need use some water treatment facilities such as sand filters for removal of fungi and bacteria in this water, especially according the regional weather conditions. Of course in this research, the rain harvested water is not used for drinking. However, this water is consumed for washing, cleaning, and other consumptions for automobile components preparation to painting. The consumed water for painting of automobile pieces in car factories should be free from of any particles of color and turbidity agents such as bacteria, fungi, algae, protozoa, and other chemical compounds such as iron, manganese, NO_3^- and some nutrients (23,25).

According to the determination of physical and chemical characteristics of entering water, no relationship is found between the parameters and microorganisms in water. In the other hand, the microorganisms are existed in some units that could be related to physical and chemical

properties such as total suspended solids and turbidity. Furthermore, considering the consumed water as chemical and microbial qualities (Tables 1-3) in the Babol car factory, it is necessary that be used water treatment facilities such as reverse osmosis system to removal of metal ions, bacteria, fungi, and protozoa in the consumed water.

Acknowledgement

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