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Investigation of the physicochemical and microbiological quality of brassware effluents: Insight into the charge of heavy metal and pollutants in wastewater from Fez, Morocco

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ABSTRACT

This article investigated the effluents from brassware wastewater in the city of Fez, Morocco. Brassware is considered one of the principal economic activities in the region, but its effluents harm the environment and human health because of its heavy metal loading. The objective of this study was to determine the physicochemical, metallic, and microbiological characteristics of the brassware effluents. The degree and nature of the pollution generated by the studied effluents from September to April 2022 were also studied. The samples were collected each month from a brassware company to evaluate the pollution using standard methods: physicochemical parameters, Temperature, pH, electrical conductivity, suspended solids, chemical oxygen demand, biological oxygen demand, sulfates, orthophosphate ions, total Kjeldahl nitrogen, nitrates, nitrites, and ammonium), metals (silver, aluminum, cadmium, cobalt, chromium, copper, nickel and lead), and microbiological, total aerobic microbial bacteria, total coliforms, and fecal coliforms, Staphylococcus aureus, Streptococcus, molds, and yeasts. The results collected during March showed that the studied effluents had a pH = 10.4 ± 0.16, electrical conductivity of 6.93 ± 0.11 mS/cm, suspended solids of 3078.15 ± 121.85 mg/L, a chemical oxygen demand of 680.44 ± 10.84 mg /L and sulfates of 1755.44 ± 21.56 mg/L, which do not correspond to Moroccan rejection standards. The metal analysis showed that the studied effluents exhibited high concentrations of nickel (999.96 ± 0.08 mg/L) and copper (76.48 ± 0.002 mg/L) during this month. Nevertheless, they were characterized by the absence of pathogenic germs. In general, the obtained results showed that these effluents were characterized by monthly variations in values for all the measured parameters. These results provide important information on the negative impact of brassware wastewater on the environment that should motivate

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municipal water utilities and researchers to find innovative solutions to this problem and protect the receiving environment.

1. Introduction

The region of Fez, Morocco, has experienced both demographic growth and a rapid evolution of its industrial sector. This development has prompted significant economic benefits for the region but has increased pressure on the use of water, resulting in a considerable degradation of its water quality [1-4]. The main industrial activities include textiles, oil mills, carpentry shops, pottery, and other craft activities, such as tanneries and brassware. In particular, the surface treatment industry or brassware industry that consists of making craft and decorative objects. It is achieved by plunging the parts into electrolytic basins (degreasing, nickel plating, copper plating, silver plating) and alternating rinsing basins. It is one of the most hazardous activities [5] because it produces a large volume of wastewater with high concentrations of toxic metals (such as copper, nickel, etc.) and other toxic elements, such as organic acids (boric acid) and cyanides [6,7], that are directly discharged into nearby watercourses. In contrast to organic pollutants, heavy metals are non-biodegradable; they persist in the environment and some may be toxic to humans, even at low concentrations [7-14]. Many studies have shown that heavy metals tend to accumulate in living tissues and organs. Consequently, they affect the function of the central and peripheral nervous, respiratory, renal, cardiovascular, endocrine, and immune systems, as well as the normal cellular metabolism [15-17]. In particular, nickel is ubiquitous in the earth at about 0.01%; it is essential in low concentrations for the function of plants and animals but can be toxic in high concentrations [18-22]. In humans, nickel and its derived compounds are responsible for lung and nose cancers and have been classified as carcinogenic by the International Agency for Research on Cancer (IARC) [23]. Copper is also present in natural aquatic environments at low concentrations (<5 µg/l); it plays an important role in the biochemistry of organisms [24,25]. At high concentrations, it causes intestinal distress, renal damage, and anemia [26]. Some previous research has focused only on the physicochemical and metallic characterization of brassware effluents in the city of Fez [6,27]. No studies in the literature have examined and monitored the quality of these discharges over time. The novelty of the present research is the study of the physico-chemical, metallic, and microbiological quality of effluents from the brassware industry in Fez to estimate the degree and nature of pollution generated each month by the most polluting artisanal activities in the region. Microbiological analysis has never been considered in previous studies. Thus, this study evaluated the actual state of the effluents for eight months and their potential risks to the receiving environment (the Sebou River) and human health to establish recommendations for their protection.

2. Materials and methods

2.1. Study site

The brassware workshop is located in the industrial district of "Ain Nokbi" in the city of Fez (Figure 1). This workshop focuses on the fabrication of artisanal pieces (teapots). Nickel and copper sulfates are used in the nickel plating and copper plating basins, and nitric acid, which is used in the stripping treatment. The wastewater of this workshop is sent directly into the receiving environment (Oued Sebou) without any preventive treatment. Consequently, such negligence causes serious problems for the city's environment.

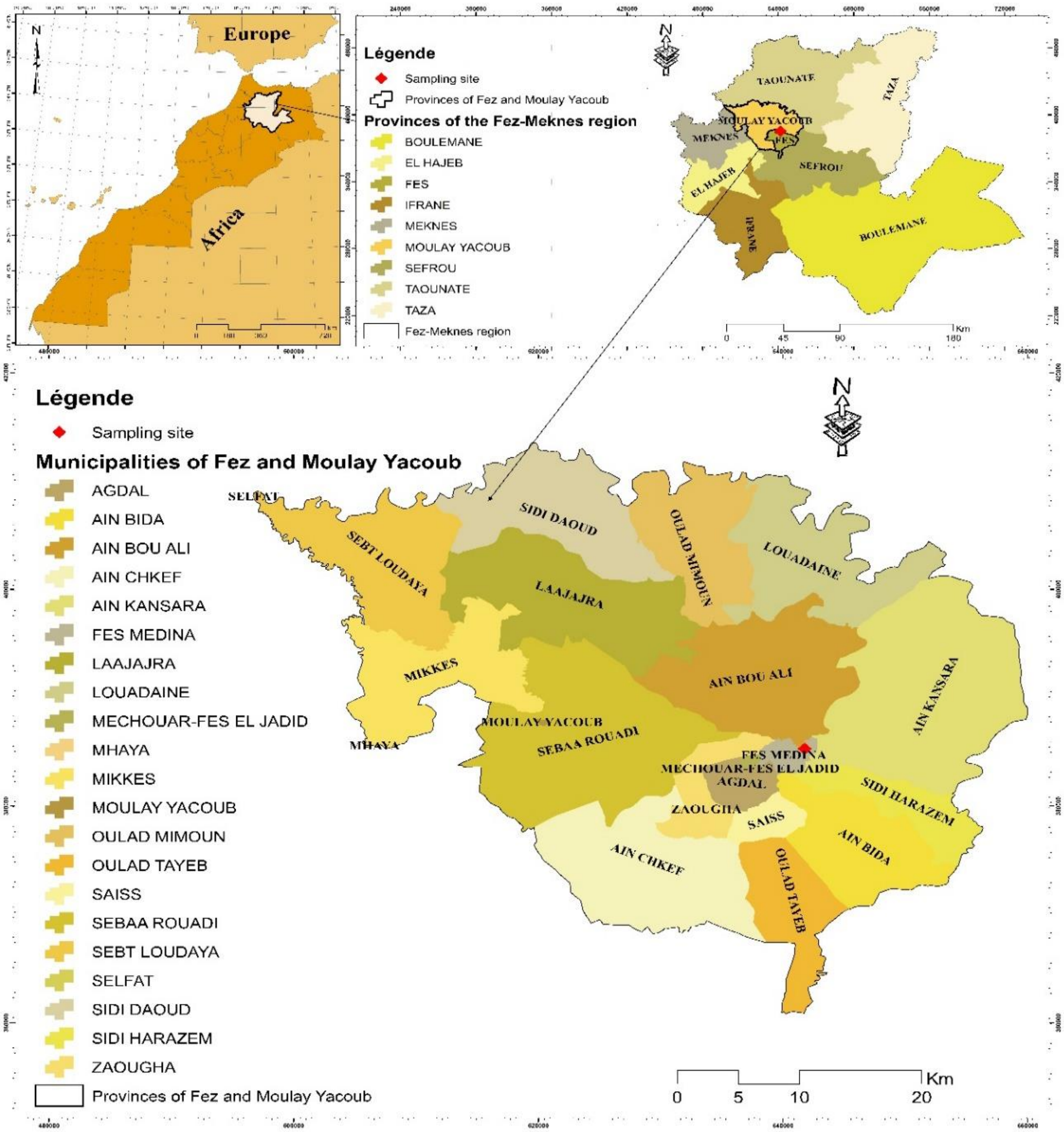
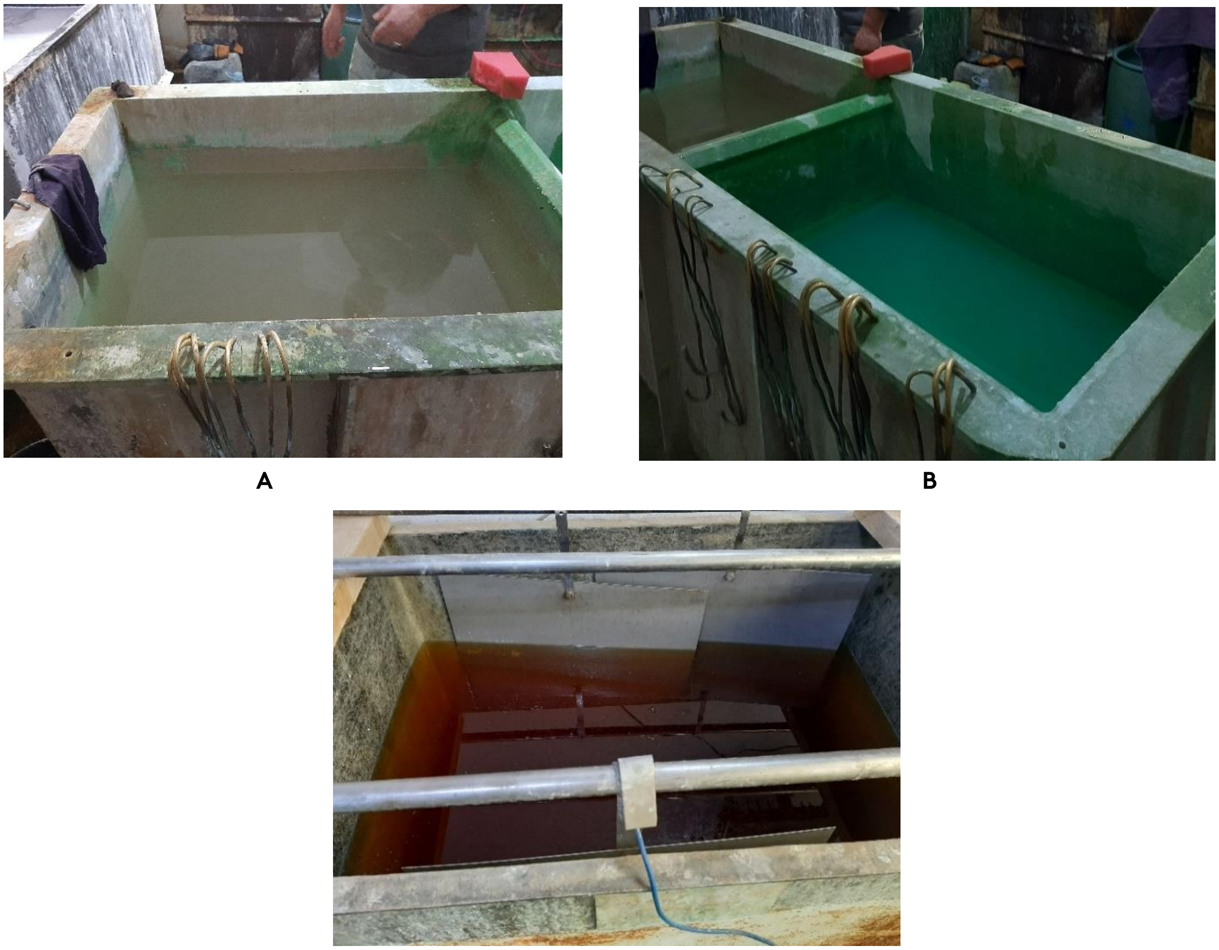


Fig. 1. Location of the study area (ArcGIS 10.4.1).

2.2. Sampling

The wastewater was collected monthly from a brassware factory from September to April 2022. The samples taken for the physicochemical analysis were gathered in polythene 2-litre tanks, pre-rinsed with water from each basin. This wastewater was composed of three types of discharges (e.g., degreasing copper plating, nickel plating, and silver plating). These samples were taken for microbiological analysis according to standard microbiological norms (homogeneity, standard, and asepsis) in 1-litre glass sterile bottles (autoclaved at 120 °C for 20 minutes) for each basin. They were labeled, hermetically closed, and stored in a cool box at 4 °C before being immediately transported to the biotechnology laboratory at the Faculty of Sciences to be analyzed within 24 hours according to the analysis methods described by Rodier [28].



A B C

Fig. 2. Photos of the three basins of the "AIN NOKBI" brassware industry in Fez, Morocco (taken on February 22, 2022): **A.** photo of the degreasing and copper plating basin. **B.** photo of the nickel-plating basin. **C.** photo of the silver-plating basin.

2.3. Physicochemical and metallic characterization

The collected samples were characterized for their temperature, pH, and electrical conductivity and measured by a multi-parameter HANNA HI98194 Instrument at 25 °C. Also, the experimental method described by Rodier was used for the suspended solids [28]. The biological oxygen demand was measured by a type of Oxi Top IS6 BOD meter at 20°C for five days. The chemical oxygen demand was determined using a COD meter (BINDER model ED 53 # 02-393770) made by AFNOR standard T90-101 [28]. The other parameters of sulfate, nitrogen compounds (Total Kjeldahl Nitrogen, nitrates, nitrites, ammonium), and orthophosphates were determined using colorimetric assays by a UV spectrophotometer type SELECTA (LIB 030 M) according to the experimental protocols [28]. Heavy metals were analyzed by the Inductively Coupled Plasma Spectrometry (ICP) method via a Horriba Jobin Yvon instrument at Sidi Mohamed Ben Abdellah University "City of Innovation."

2.4. Microbiological characterization

The wastewater obtained from the three basins was mixed with the same volume in a sterile bottle in aseptic conditions for microbiological characterization. Then, the wastewater was characterized within 24 hours by surface inoculation of 0.1 mL, with a series of dilutions from 10^{-1} to 10^{-6} performed in three repetitions according to the experimental protocols described by Rodier [28]. The germs, culture medium used, time, and temperature of incubation are shown in Table 1.

Table 1. Germs and culture medium used.

Germs	Culture medium used	Time and temperature of incubation
Total aerobic mesophilic bacteria (TAMB)	LB agar (Luria Bertani)	24 to 48 hours at 30 °C
Total coliforms (TC)	Desoxycholate Lactose agar	24 hours at 37 °C
fecal coliforms (FC)	EMB agar (Eosin methylene blue)	24 hours at 44 °C
Yeasts	Yeast-extract-Peptone-Glucose-Agar	3 to 5 days at 30 °C
Molds	Malt Extract Agar	3 to 5 days at 30 °C
Staphylococcus aureus	Chapman-mannitol agar	24 hours at 37 °C
Streptococcus	Slanetz and Bartley agar	24 hours at 37 °C

2.5. Statistical analysis

All the experiments were repeated three times. The significance of the difference between the means was tested by analysis of variance (ANOVA). Tukey multiple range tests at $p<0.05$ were performed using GraphPad Prism 7 software.

3. Results and discussion

3.1. Physio-chemical characteristics

Figures 3, 4, 5, and 6 show the results of the physicochemical characterization of the brassware's effluents studied over eight months. According to Figure 3A, a slight temperature change was observed during this study period, with a maximum temperature of 21.87 °C ± 0.06 registered in November and a minimum temperature of 16 °C ± 0.16 in December. These temperature variations may be attributed to air temperature variations [29]. Moreover, the pH of the effluents during the study period was slightly alkaline (Figure 3B) and varied between 9.22 ± 0.025 and 10.4 ± 0.16. However, they surpassed the rejection standards for Morocco (5.5-8.5) [30]. The value obtained for pH was due to the excessive usage of sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), and sodium cyanide, which were used in the degreasing basin to remove all traces of grease from the produced pieces. In addition, the optimal pH values for microorganisms' survival varied between 6.5 and 7; thus, the highly alkaline pH affected the environmental conditions and could reduce the survival of the microorganisms [31]. Despite the obtained outcomes, herein being quite different from those previously described by Laidi et al. and Sarkar et al. [6,32] at a neutral pH of 7.43, they are similar to those obtained in previous tannery wastewater studies [33-36] [33–36]. Electrical conductivity has been studied as an indicator of pollution from wastewater discharges [37]. From Figure 3C, we can observe that the averages of the electrical conductivity are superior to the Moroccan rejection standard (2.7 mS/cm) [30]. They fluctuated from 3.45 ± 0.06 $mS.cm^{-1}$ to 6.93 ± 0.11 $mS.cm^{-1}$, the latter registered in March. In addition, the averages of September and April were comparable at (p = 0.9900); the averages of October and January are also comparable at (p = 0.9980). These reflect the extensive usage of salt, i.e., copper salts, cyanides, silver potassium, and nickel sulfates, in the surface treatments. Elsewhere, the recorded values for electrical conductivity were different from those found in previous work [38]. Water containing high levels of minerals had a high conductivity, and when this was above 3 $mS.cm^{-1}$, conditions unfavorable to normal ecological equilibrium occurred due to moderate to severe pollution [39,40]. Orthophosphate ions were present in low concentrations, ranging between 1.93 ± 0.012 mg/L and 9.28 ± 0.07 mg/L detected in September and

March, respectively (Figure 3D). The averages for September, October, and February were rather similar, respectively ($p = 0.9314$), ($p > 0.9999$), and ($p = 0.8426$). The mean values of this last parameter were also inferior to the Moroccan discharge standard (15 mg/L). These results can be explained by the absence of detergents in the degreasing or rinsing tanks [6]. The concentrations of ortho-phosphate ions measured were comparable to those found in previous research (88.06 mg/L) [41] and inferior to those cited in another research (30.12 mg/L) [42-43]. Industrial effluents result in a significant increase in the organic load in the receiving environment. This is reflected in the variable consumption of O_2, causing variations in BOD_5 and COD [39]. The mean values of chemical oxygen demand in the effluents ranged from 515.67 ± 9.33 mg O_2/L^- to 680.44 ± 22.88 mg O_2/L. However, they were significantly higher than the biological oxygen demand, with a value maximum of 22.91 ± 0.28 mg $O_2.L^{-1}$ recorded in December (Figure 4). This could be explained by workers using more chemical products in their workshops. Extremely high COD and BOD_5 concentrations could be caused by the abundance of organic matter. The wastewater from this industry has a low content of biodegradable organic matter in terms of BOD_5. On the one hand, tanning wastewater is highly contaminated with organic and metallic elements [36]. Furthermore, the measured COD concentrations were very low compared to the studies performed by other researchers [6,27,32,44] and, therefore, lower than other studies [45-46]. On the other hand, the results for BOD_5 were the same as those cited in the literature [6,46]. It should be noted that the suspended solids are all the mineral and organic particles present in the wastewater. Their influence on the physicochemical characteristics of the water is very harmful[47-48]. A monthly variation of suspended solids was observed, with values ranging from 1369.3 ± 2.66 mg/L to 3078.15 ± 121.85 mg/L (Figure 5), whereas the results obtained for November and January were significantly comparable ($p = 0.1129$). In addition, they were largely different from the Moroccan rejection standard (100 mg/L) [30]. These could be justified by the dissolution of the chemicals and the stripping of the parts during the surface treatment. On the one hand, the values recorded in the present study were similar to those of Jeganathan et al., Rassam et al., Chaouki et al., and Shekhar et al. [46,49]. Furthermore, very high levels of sulfates ions were present in the studied wastewater, exceeding the established Moroccan standard of 600 mg/L [30]. High-level concentrations of sulfates ions were observed in March (1755.44 ± 21.56 mg/L). However, very low concentrations were recorded (898.583± 9.45 mg/L) during February. In addition, March and April were characterized by almost similar values ($p = 0.1061$). These values were probably due to the use of sulfates products, namely nickel and copper sulfates, in their respective plating tanks (Figure 5). These results were rather low compared to a study by Elkarrach et al. [34] and much higher than those described by Doumbi et al. [36]. The nitrogen forms, ammonium, nitrite, and nitrate concentrations of the brassware were also characterized in this study. The results revealed that these parameters did not register any notable maximum concentration values (Figure 6). These results were different from a research study by Aregu et al. [43]. The concentrations of nitrates varied between 1.26 ± 0.13 mg/L and 3.87 ± 0.01 mg.L^{-1}, and were also inferior to those obtained by previous studies during the characterization of tannery wastewater [41,49]. Concerning the total Kjeldahl nitrogen, a monthly variation occurred with the lower concentration (8.38 ± 0.146 mg/L) recorded in September and the higher concentration (15.76 ± 0.46 mg/L) in March. They originate from the nitric acid that is applied in the stripping treatment; it acts by chemically dissolving the metal surface. In addition, the values for all the parameters, namely NTK, nitrates, and ammonium, were well below the Moroccan discharge standard (40 mg/L).

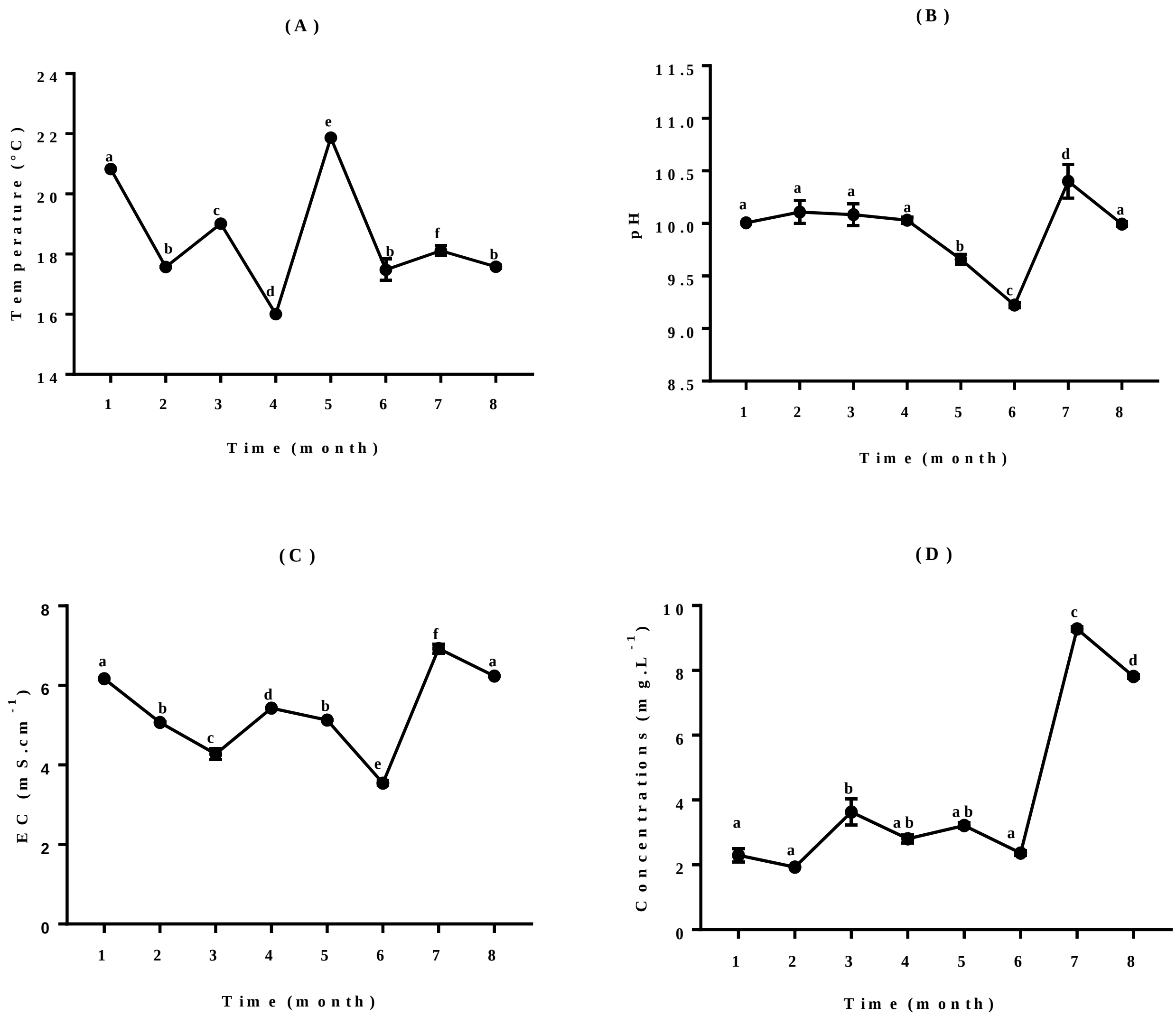


Fig. 3. Monthly variation of temperature, pH, electrical conductivity and Ortho-phosphate: **A.** monthly temperature variation of raw brassware effluent; **B.** monthly variation of pH of raw brassware effluent; **C.** monthly variation of electrical conductivity of raw brassware effluent; and **D.** monthly variation of ortho-phosphates in raw brassware effluent. Means (± SD, n=3) indicated by the same letters indicate no significant difference in the concentrations of each parameter between months according to multiple Tukey range tests at $p<0.05$.

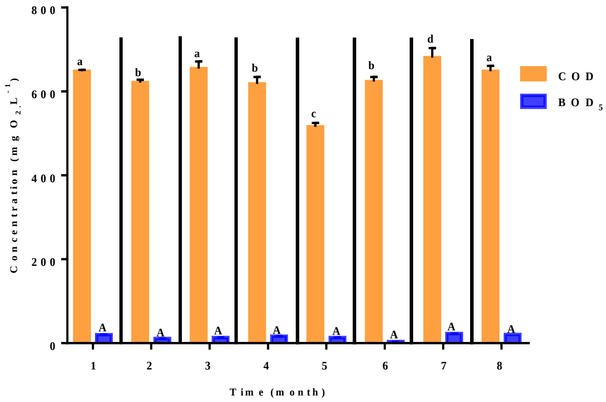


Fig. 4. Monthly variation of chemical oxygen demand and biological oxygen demand of raw brassware effluent. Means (± SD, n=3) indicated by the same small and capital letters indicate no significant difference in the concentrations of each parameter between months according to multiple Tukey range tests at $p<0.05$.

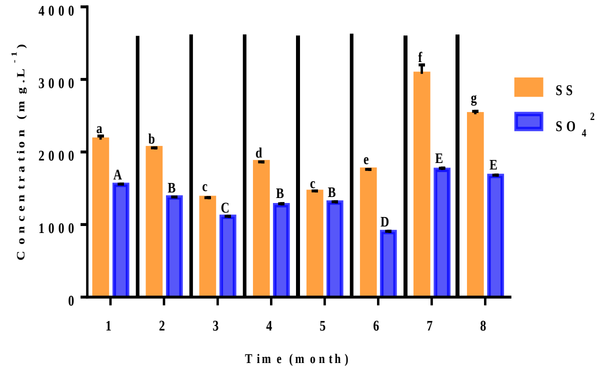


Fig. 5. Monthly variation of suspended solids and sulfates of raw brassware effluent. **SS:** suspended solids; **SO_4^{2-}:** Sulfates. Means (± SD, n=3) indicated by the same small and capital letters indicate no significant difference in the concentrations of each parameter between months according to multiple Tukey range tests at $p <0.05$.

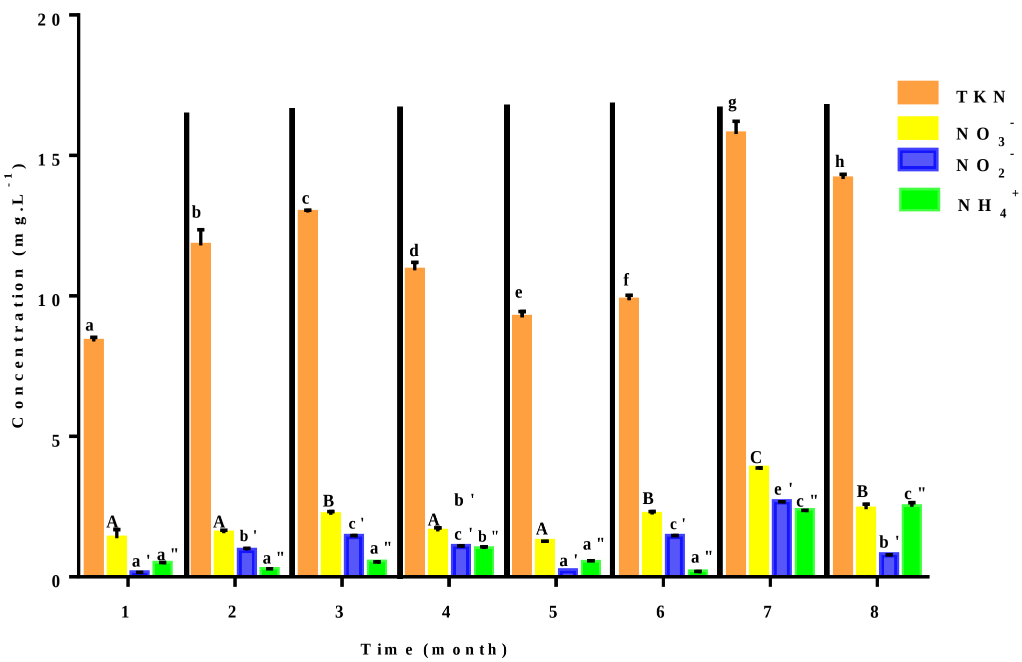


Fig. 6. Monthly variation of nitrogen compounds of raw brassware effluent. TKN: Total Kjeldahl Nitrogen; NO_3^-: Nitrates; NO_2^-: Nitrites; NH_4^+: Ammonium. Means (± SD, n=3) indicated by the same small and capital letters with one prime and two primes indicate no significant difference in the concentrations of each parameter between months according to multiple Tukey range tests at $p<0.05$.

3.2. Characteristics of heavy metals

According to the results illustrated in Table 2, the wastewater from the brassware was characterized by metal loads, which were significantly higher than the Moroccan standards. The major heavy metals recovered in the effluent were nickel, copper, and silver; very high concentrations of nickel and copper (999.96 ± 0.08 mg/L) and (76.48 ± 0.02 mg/L), respectively, were recorded in March. The less important silver concentrations varied between (0.31 ± 0.01 mg/L) and (4.17 ± 0.02 mg/L). In contrast, the lead concentrations were very minimal and almost the same, except for a high concentration (1.99 ± 0.04 mg/L) detected in March. This concentration exceeded the Moroccan standard (1 mg/L). But for all other elements, aluminum, cadmium, chromium, and cobalt were present at very low concentrations (0.01 mg/L) and respected Moroccan standards. The obtained values were comparable to those of the reported study [6]. These elements resulted from the utilization of copper salts, nickel chlorides ($NiCl_2$ $_6H_2O$), nickel sulfates ($NiSO_4$ $(H_2O)_6$), and silver cyanide during the copper, nickel, and silver-plating processes. In particular, the monthly variation in heavy metal concentrations was strongly related to the quality and quantity of the used products for surface treatment. Furthermore, these results indicated that heavy metal pollution poses a serious problem for living organisms in the aquatic receiving environment [50].

3.3. Microbiological characteristics

The microbiological analyses performed on the raw brassware effluent were repeated three times. They showed a monthly variation of the total aerobic mesophilic flora germs with a significant concentration of 6.64 $.10^2$ ± 4.51 $CFU.ml^{-1}$ recorded in November and a low concentration registered in March. It could be noted that this low concentration was related to the high concentrations of nickel and copper recorded in March compared to those recorded in November. In addition, they indicated an absence of pathogens, in particular total coliforms, fecal coliforms, *fecal Streptococcus*, *Staphylococcus aureus*, yeasts, and molds (Table 3). It can be concluded that these low concentrations could be mainly due to the toxicity of salts and heavy metal contaminants that affected the growth of bacteria and reduced the microbial biomass [11,51]. We can also conclude that these FMAT germs were resistant to high concentrations of heavy metals, including nickel, copper, silver, and salts, and they are known as halophilic bacteria [52]. Our results were similar to those cited in the literature [34]. According to these microbiological results, the industrial wastewater of the brassware did not hurt the receiving environment because this microbial charge respected Moroccan standards [30].

Table 2. Monthly variation of heavy metal concentrations in the brassware effluent.

Time (month)	Concentrations of metallic elements (mg/L)							
	Ag	Al	Cd	Co	Cr	Cu	Ni	Pb
September	3.156 ± 0.04 a	0.01 ± 0 A	0.01 ± 0 a	0.01 ± 0 A	0.01 ± 0 a	12.21 ± 0.18 a	105.93 ± 0.3 a	0.06 ± 0 a
October	3.54 ± 0.13 b	0.01 ± 0 A	0.08 ± .10 a	0.01 ± 0 A	0.01 ± 0 a	60.23 ± 0.09 b	23.42 ± 0.27 b	0.01 ± 0 a
November	1.508 ± 0.03 c	0.01 ± 0 A	0.06 ± .01 a	0.01 ± 0 A	0.01 ± 0 a	0.27 ± 0.03 c	42.48 ± 0.50 c	0.04 ± 0 a
December	1.22 ± 0.10 d	0.01 ± 0 A	0.01 ± 0 a	0.01 ± 0 A	0.01 ± 0 a	24.09 ± 0.02 c	18.19 ± 0.13 d	0.01 ± 0 a
January	0.31 ± 0.01 e	0.01 ± 0 A	0.01 ± 0 a	0.01 ± 0 A	0.01 ± 0 a	4.28 ± 0.02 e	94.05 ± 0.19 e	0.01 ± 0 a
February	2.53 ± 0.09 f	0.01 ± 0 A	0.01 ± 0 a	0.01 ± 0 A	0.01 ± 0 a	30.34 ± 0.21 f	64.44 ± 0.12 f	0.13 ± 0.01 a
March	4.17 ± 0.02 g	0.01 ± 0 A	0.01 ± 0 a	0.01 ± 0 A	0.01 ± 0 a	76.48 ± 0.02 g	999,96 ± 0.08 g	1.99 ± 0.04 b
April	3.12 ± 0.01 a	0.01 ± 0 A	0.01 ± 0 a	0.01 ± 0 A	0.01 ± 0 a	45.76 ± 0.23 h	60.61 ± 0.1 h	0.52 c
Moroccan discharge standards (mg/L)	**0.1**	**10**	**0.25**	**0.1**	**2**	**2**	**5**	**1**

Ag: Silver; **Al**: Aluminum; **Cd:** Cadmium; **Co**: Cobalt; **Cr:** Chromium; **Cu:** Copper; **Ni**: Nickel; **Pb:** lead. Means (± SD, n=3) followed by the same letters in the same column indicate no significant difference in the concentrations of each parameter between months according to multiple Tukey range tests at $p<0.05$.

Table 3. Monthly variation of germs concentrations in the brassware effluent.

Time (month)	Germs CFU.ml^{-1}						
	TAMB	Fecal coliforms	Total coliforms	*Streptococcus*	*Staphylococcus aureus*	molds	yeasts
September	4.28.10^2 ± 13.32 a	Abs	Abs	Abs	Abs	Abs	Abs
October	4.91.10^2 ± 3.52 b	Abs	Abs	Abs	Abs	Abs	Abs
November	6.646.10^2 ± 4.51 c	Abs	Abs	Abs	Abs	Abs	Abs
December	4.067. 10^2 ± 4.73 d	Abs	Abs	Abs	Abs	Abs	Abs
January	5.473. 10^2 ± 4.51 e	Abs	Abs	Abs	Abs	Abs	Abs
February	5.046. 10^2 ±11.51 f	Abs	Abs	Abs	Abs	Abs	Abs
March	3.267. 10^2 ± 7.03 g	Abs	Abs	Abs	Abs	Abs	Abs
April	3.796. 10^2 ± 7.09 h	Abs	Abs	Abs	Abs	Abs	Abs

TAMB: Total aerobic mesophilic flora bacteria. Mean values (±SD, n=3) followed by different letters in the same line are significantly different in the concentrations of each germ between months according to multiple Tukey range tests at $p<0.05$. **Abs:** indicated absence of germs.

4. Conclusions

This study was carried out to examine the physico-chemical, metallic, and microbiological state of effluents from brassware in the city of Fez, and to monitor the degree of pollution over eight months. It can be concluded that the levels and concentrations of certain parameters, such as suspended solids, chemical oxygen demand, electrical conductivity, and sulfates, did not comply with the Moroccan discharge standards. In addition, they were characterized by a very low microbial charge (TAMB) and very high concentrations of heavy metals, notably nickel, copper, and silver. The results demonstrated that in March, significantly higher concentrations of physicochemical and metallic characteristics were registered than in other months, followed by a low microbial charge. Consequently, this confirmed that the effluents generated by the brassware were not biodegradable and persistent. They pose a serious threat to human health and are a source of pollution to agriculture, thus presenting a risk to the biodiversity of the Sebou River. The Sebou basin is very important in the Moroccan kingdom, which currently has a total population of 6.3 million. In this regard, it is necessary to develop a treatment method appropriate for this type of effluent, established by strict surveillance and an effective control program to minimize the environmental risks for the Sebou River.

Authorship contribution statement

Imane ZOUFRI: Writing-original draft, writing-review and editing, methodology, conceptualization.
Mohammed Merzouki: Validation, Supervision.
Malika Ammari: Formal Analysis, Methodology, Writing-original version.
Younesse EL-BYARI: Investigation, Methodology.
Amina BARI: Conceptualization, Supervision.

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Competing interest

The authors declare no conflict of interest.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

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