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# Quantitative Determination of Phthalate Esters and Bisphenol-A Residues in Wastewater Treatment Plants Outflow in Saudi Arabia: Gas Chromatography/Mass Spectrometry-Based Analytical Study

**Ezat A. Mersal [1, 2*], Ahmed A. Morsi [3], Shaimaa Alakabawy [4], Riham G Elfawal [4, 5], Eman M. Sakr [2, 6], Ahmed M. Abdelmoneim [7], Ahmed H. Ahmed [8], Saad Alswiahib [9], Fatimah Al Salem [9], Tamer Mohamed Shawky [10]**

[1] *Biochemistry Department, Faculty of Science, Assiut University, Assiut, Egypt*
[2] *Department of Basic Medical Sciences, Vision College of Medicine, Vision Colleges in Riyadh, Saudi Arabia*
[3] *Histology and Cell Biology Department, Faculty of Medicine, Fayoum University, Fayoum, Egypt*
[4] *Department of Clinical of Sciences, Vision College of Medicine, Vision Colleges in Riyadh, Saudi Arabia*
[5] *Clinical Pathology Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt*
[6] *National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt*
[7] *Physiology Department, Faculty of Medicine, Fayoum University, Fayoum, Egypt*
[8] *Department of Basic Sciences, Vision College of Dentistry and Nursing, Vision Colleges in Riyadh, Saudi Arabia*
[9] *Students at Vision College of Medicine, Vision Colleges in Riyadh, Saudi Arabia*
[10] *Department of Anatomy and Embryology, Faculty of Medicine, Cairo University, Giza, Egypt*

**Abstract**

The aquatic system is unfortunately vulnerable to contamination by chemicals as bisphenol A (BPA) and phthalate esters (PAEs) that arise from plastic products frequently used. Water supply is limited in Saudi Arabia. Therefore, many wastewater-treatment plants (WTPs) have been established for wastewater recycling in industry and agricultural purposes. The current study updated and validated the concentrations of BPA and six PAEs (dibutyl phthalate (DBP), dimethyl phthalate (DMP), diethyl phthalate (DEP), butyl benzyl phthalate (BBP), bis (2-ethylhexyl) phthalate (DEHP), and dioctyl phthalate (DOP)) in wastewater samples obtained from 5 wastewater treatment plants in 3 Saudi cities. Also, the study compared the concentrations of these chemicals in the secondary WTPs versus the tertiary ones. Chromatography/mass spectrometry was used for the extraction of the chemicals in water samples. All measurements were exposed to comparison and correlation statistical analysis. Both DEHP and DBP were found in all obtained treated wastewater samples. Meanwhile, DOP, BBP, DEP, DMP, and BPA were identified in the following percent of samples, 74.1%, 80.2%, 84%, 84.1%, and 98.01% respectively. The levels of DMP ($p < 0.05$), DOP, ($p < 0.05$) and BPA ($p < 0.05$) were higher in tertiary-treated water sample than those secondary.

Keywords: Aquatic system; wastewater treatment; water pollution; environmental sciences; agricultural irrigation.

## 1. Introduction

Water is vital for survival on Earth. Unfortunately, it is also a vulnerable and finite resource with qualitative and quantitative constraints. By the year 2025, it is estimated that 3.5 million individuals worldwide may face a water shortage problem. Globally where freshwater is deficient, wastewater is used frequently for irrigation of lands and crops [1]. Wastewater production includes various sources such as municipal, agricultural, and industrial activities. These sources are often polluted with different organic and inorganic contaminants as

*Corresponding author e-mail: mersalezzat@gmail.com.; (Ezat A. Mersal).
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microplastics, heavy metals, xenobiotics, …etc which negatively affect the food chain and thus transmitted and threatened human life mainly via plant uptake of the pollutants [2]. In addition, crop irrigation by wastewater might lead to a change in the physical, chemical, and microbiological characteristics of the soil with subsequent soil hardening. Moreover, the main drawback of crop irrigation by wastewater is the presence of endocrine disrupting chemicals (EDCs) as one of the common contaminants of the wastewater [3].

Saudi Arabia is one of the countries that have paucity in of water resources. Governmental efforts were directed to the settlement of many numerous wastewater treatment plants (WTPs) for further reuse of water in land irrigation and industry [4].

Bisphenol-A (BPA), Phthalate esters (PAEs), and other contaminants are common EDCs that have been detected in treated wastewater [5,6], despite the governmental efforts carried out to minimize them during wastewater treatment.

Phthalate esters (PAEs) are frequently found in daily handled products, and are used as plasticizers in industry, particularly in polyvinyl chloride (PVC) products [7]. Dibutyl phthalate (DBP), dimethyl phthalate (DMP), diethyl phthalate (DEP), butyl benzyl phthalate (BBP), bis (2-ethylhexyl) phthalate (DEHP), and dioctyl phthalate (DOP) are commonly occurring PAEs [4,8]. On the other hand, BPA is an intermediate compound in the production of polycarbonates, polystyrene, and epoxy resins [9]. The high durability of plastics and their continuous use raised the environmental concerns about a novel sort of pollution named micro plastic pollution. Due to the non-covalent bonding to plastic, these pollutants frequently leach from the daily used product into the air, food, water, and nearby environment [10]. Hence, people are simultaneously exposed to not merely phthalates, but also other EDCs [11], that is why the Hazard Index for phthalate mixture exposure has been recommended to be reduced [12]. Several studies [13,14] have indicated that these compounds can exert an additive effect when applied in a mixture.

A former study [15] has declared that water treatments are not efficient to extract these chemicals from wastewater. The use of treated wastewater for irrigation purposes in countries having poor freshwater resources, like KSA is very important however, the filtering efficiency of WTPs in separating pollutants with high risk to public health is a great concern raised by the inhabitants of the country. Appropriate quality evaluation after water treatment and before its use in agriculture can change the thoughts and perceptions of the general people regarding such issues.

A previous study conducted by Al-Saleh et al. [4] measured the concentrations of PAEs and BPA, in 5 Saudi WTPs. Therefore, the work aimed to validate and update the measurements of BPA and the six PAEs concentrations in 250 secondary- and tertiary-treated wastewater samples obtained from the 5 WTPs in 3 Saudi cities using the chromatography/mass spectrometry analytical method. Also, the study assessed different filtration technologies through evaluating the efficacy of secondary versus tertiary treatment plants by comparing the concentrations of these chemicals in the treated wastewater samples obtained from each.

## 2.Material and methods

### 2.1. Water sampling and extraction procedure of Bisphenol A & phthalates esters

Five replicates of treated wastewater were collected weekly from five wastewater treatment plants in three main Saudi cities (Riyadh, Ehssa & Taif). All samples underwent tertiary processing (treatment) apart from the ones collected from Manfouha and Wadi Hanifah WTPs. Both secondary and tertiary treatment plants included biological treatment (using microorganisms), meanwhile sand filtration is added as an advanced treatment in tertiary ones. 250 samples were collected for five consecutive weeks; 150 samples from Riyadh (50 tertiary treated water from King Saud University's treatment plant, 50 binary treated waters from Wadi-Hanifah, and 50 binary treated water from the major pump in Manfouha), 50 samples were collected from Ehsaa (tertiary treated from Al-Hofuf pump) and 50 samples from Taif (tertiary treated from The National Water Company in Wadi-Alarj). Glass bottles were washed with water (deionized) followed by methanol washing and were placed for one hour in an oven (100 °C). The bottles, containing the collected samples, were sealed with aluminum foil then kept refrigerated (4 °C) till analysis.

Analysis of diethylphthalate (DEP), di-n-butyl phthalate (DBP), diethyl hexyl phthalate (DEHP), Dimethyl phthalate (DMP), benzyl butyl phthalate (BBP), and di-n-octyl phthalate (DOP) in 250 samples of treated wastewater was performed by using headspace solid-phase micro-extraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS) as described by Al-Saleh et al [4].

### 2.2. Chemical analysis:

#### 2.2.1. Bisphenol-A

BPA was extracted and analyzed as previously mentioned by the method of Szyrwinska et al. [16] with minor modifications. Every water sample was treated with an IS (40 µg/L d16-BPA) and was dried at 60 °C using nitrogen gas evaporation. The residue was combined with the BSTFA derivatization agent (containing 1% TMCS) and vortexed, then the bottles were kept in an $H_2O$ bath at 75 °C for one hour. The samples were dried again after cooling and suspended in chloroform (1mL). Standard Calibration was prepared in the same way, using a stock solution of 1µg/ml and a calibration range of 5–160µg/L. BPA and d16-BPA stock solutions (1µg/mL) were prepared in methyl alcohol. Gas Chromatograph (Agilent model 7890A) coupled to Mass Selective Detector (Agilent 5975C) with triple-axis detector and auto injector (7693A) was used. In a mass range (30–600 m/z), the detector was adjusted (70 eV electron energy) in the

mode of electron impact (EI) ionization. At a flow rate of 0.8ml/minute, highly pure helium (99.999 %) was being used as a gas carrier. Fused-silica capillary column (Apigent J&W DB-5MS), was utilized for chromatographic separation. The initial column temperature was 80 °C, which was kept for half minute, followed by an increase (20 °C/minute) up to 250 °C, in the first run, and a 20 °C/minute increase in the second run to 290 °C, which was held for 6 minutes. 2 µL of the sample were inserted into the Gas Chromatograph-Mass Selective Detector. The total duration of the performance was: 17 minutes. The ion source had a temperature of 230 °C. Bisphenol-A was quantified using SIM (m/z of 357 for target ions), 372 and100 (as qualifier ions). For the IS (d16-BPA), the ions used were ions used were those quantified at m/z 368 and 386.

### 2.2.2. Phthalate esters

A gas chromatograph (6890N) coupled to a quadrupole mass-selective spectrometer (GC-MS), model 5973 (Agilent, Palo Alto, USA) was used to analyze PAEs. The acquired data were analyzed by Chemstation software (G1701 DJ, Agilent). The column was an Agilent J&W DB-5MS capillary column. The injector was made with an Agilent inlet liner (4 mm i.d.) packed with glass wool. A CombiPAL autosampler with a cooler tray, a 32-sample tray, a temperature controller, an SPME fiber holder, and a 20-ml-vial agitator was provided for the GC–MS. The A 65 µm PDMS/DVB fiber was chosen based on the recommendation of Carrillo et al. [17] and Alshehri et al. [18]. The temperature of the GC oven was set to rise at a rate of 10 ° C/ minute from 80 ° C (kept for 0.5 minute) to 220 ° C, then to 290 ° C at 30 ° C (held for 4 minutes). Perfluorotributylaminutee was used to auto-tune the MS. Electron ionization (EI) was carried out at 70 eV. The ion supply was calibrated to 250°C, with a 99.999 % pure helium carrier gas flow rate of 1 mL/minute. The injections were conducted through splitless mode (held for 2 minutes), with a split flow of 50 mL/minute at a temperature of 300 °C for the injector. With an eight-minute solvent delay, the MS system was adjusted to SIM mode (selected ion monitoring). Quantitative measurements were also made using the SIM mode. For each chemical, three fragment ions were measured. The fragments were selected, and their retention durations were determined after injecting standard solutions in full-scan mode. For quantification, the most characteristic ion in the spectrum was chosen, and the other two ions were chosen for confirmation. For quantification, the areas of the peaks were employed. The PAEs were measured and expressed as µg/L. Ten ml of the sample was spiked with 6 µl of DPrP-d4 as an IS (2 g/µg/L) and was put in a 20-ml glass vial for each SPME assay (75.5 mm long and 22.5 mm in diameter). A 1.5-mm PTFE/silicone septum was then used to tightly seal the vial. The samples were thoroughly mixed before being left to equilibrate for 10 minutes at 40 °C. The sample vial was placed in the agitator of the CombiPAL autosampler. SPME was performed at 90 °C for 13 minutes with a 500 rpm agitation rate. The analytes were thermally released from the SPME fiber and introduced into the GC–MS inlet after 5 minutes at 270°C. The optimum time of the extraction for almost all PAEs was 30 minutes. Four replicates of each water sample were evaluated.

Dissolving 25 mg of each phthalate in 25 mL of dichloromethane was used to prepare the initial stock standard solutions. In methanol, a standard solution of mixed PAEs (DEP, DMP, BBP, DBP, DOP, and DEHP) was produced, including 1.5 µg/L of every compound. Both stock/intermediate spiking standards were kept at 4 °C until they were used. The PAEs were found in quantities ranging from 0.75 to 24µg/L. Also,1.2 µg/L was obtained, as a final concentration, by adding IS. There was also a blank (plain) standard with just water (deionized) and the IS. Within 19 minutes, the six PAEs and the IS were evaluated.

## 3. Statistical analysis

Statistical analysis of the collected data was performed by SPSS software, version 24 (IBM SPSS Statistics for Windows, Armonk, NY, USA). The concentrations of BPA and PAEs were presented as mean ± standard deviation, median, minimum, and maximum. The levels of BPA, PAEs, and the weekly sampling were analyzed using One-way ANOVA. Post hoc Tukey's test was used to determine statistical significance among means. The PAEs and BPA concentrations in both secondary and tertiary wastewater treatment were compared using unpaired Student's t-test at confidence level of 95%. Before analysis, initial check of normal distribution was done using Kolmogorov–Smirnov test. The degrees of freedom (DF) were equal to 248 for each sample.

## 4. Results

### 4.1. Limit of detection (LOD) and limit of quantification (LOQ)

The calibration of GC–MS and SPME–GC–MS was assessed by calculating the LOD and LOQ. The LOD was calculated as LOD = blank mean + 3 (standard deviation) and LOQ = blank mean + 10 (standard deviation). The results of 10 analyzed blank (standard) solutions, at levels of 0.375 µg/L for PAEs and 3.75 µg/L for BPA, were used to calculate the LOD and LOQ. At three concentration levels for BPA (7.5, 15, and 30 µ g/L) and each PAE (1.125, 2.5, and 4.5 µ g/L), the validation of LOQ (precision) was calculated as relative standard deviation (RSD). Both the PAEs and the BPA had good recoveries. The RSDs for both within-run and between-run precision were more than or equal to 10. Thus, the SPME-GC-MS method was precise and accurate for the quantitative

determination of the designated phthalate in treated wastewater samples. Hence, both methods were accurate when used to determine BPA and the six PAEs levels in treated wastewater specimens.

### 4.2. Detection of BPA and PAEs in treated wastewater

The detection of BPA and PAEs in treated wastewater samples was done using chromatographic analysis (fig. 1). The concentrations of BPA and the six PAEs in the wastewater samples collected from the 5 WTPs were displayed in Table 1. The findings of the seven chemicals in all measured water samples were provided in excel sheet (supplementary material 1). BPA and six PAEs were detected in the treated wastewater samples (n=250) at concentrations ranging between the LOD and LOQ. The average concentration of BPA was 4.44 µg/L with the highest concentration from Wadi Al-Araj (6.8 µg/L) while the lowest one was an average of 3.6 µg/L from Wadi Hanifah. Regarding the six PAEs that were measured in the same five WTPs samples, their average concentrations were: 0.19 µg/L, 0.5µg/L, 0.25 µg/L, 0.25 µg/L, 0.39 µg/L, and 0.8 µg/L for DEP, DEHP, DMP, DOP, BBP, and DBP respectively. The greatest and least concentration of DEHP was found in Wadi Al- Araj and King Saud University (0.94 µg/L and0.35 µg/L) respectively. DMP concentrations ranged from 0.08 µg/L to 0.47 µg/L across the five WTPs. Moreover, DOP concentrations were negligible in King Saud University WTP (0.07 µg/L) and maximum in Manfouha WTP (0.57 µg/L). DEP average concentration was the lowest among the six PAEs (0.19 µg/L). Its upper and lower limit concentrations were detected in Wadi Hanifah and King Saud University WTPs (0.0.31 µg/L and 0.12µg/L) correspondingly. DBP measured concentrations were the highest in Manfouha (1.49 µg/L) and the smallest in Wadi Hanifah(0.39 µg/L). The average concentration of DBP is the highest among the six PAEs (0.8 µg/L). Finally, BBP significant concentration was determined at Wadi Hanifah (0.7 µg/L), while the lowest was for Wadi Al- Araj(0.12 µg/L). The frequencies of sample detection above the LOQs of 0.786 µg/L (DBP), 0.945 µg/L (BBP), 0.356 µg/L (DMP), 0.761 µg/L (DEHP) and 4.646 µg/L (BPA) were 64 (25.6%), 10 (4%), 12 (4.8%), 23 (9.2%) and 74 (29.6%) respectively.

### 4.3. Difference in the levels of BPA and PAEs between treatment plants

There was a significant variation in the amounts of the six PAEs and BPA in the treated wastewater samples obtained from the five WTPs (Table 1). Tukey's post hoc test was used to do multiple comparisons between the PAE and BPA levels for the five WTPs. DEHP and DBP levels were significantly higher ($p<0.05$) in Manfouha and Wadi Al-Araj than in the other WTPs. In Manfouha and Wadi Hanifah, the similar pattern was seen for BBP and DOP p ($<0.05$). DEP levels varied significantly amongst plants, with the greatest levels in Manfouha, the lowest in Wadi Hanifah, and moderate levels in Wadi Al-Araj and King Saud University water plants.

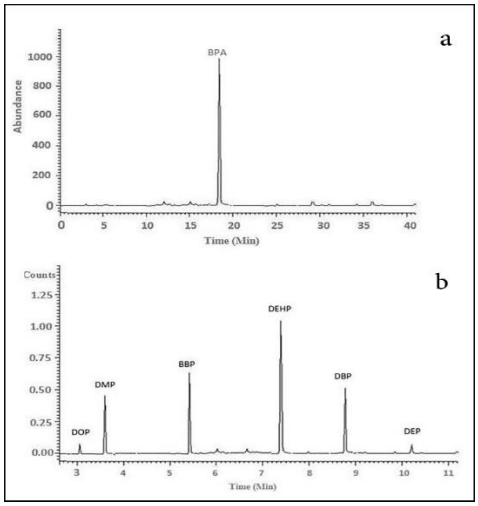


**Fig.1** Chromatograms of BPA (a) and six phthalate esters (b) detection in treated wastewater samples.

### 4.4. Effect of treatment on BPA and PAEs concentrations

A Student's t-test was used to examine the differences in PAEs and BPA levels between the 2 treatment plants (secondary and tertiary). Secondary-treated samples had considerably greater DBP ($p<0.05$) and BBP ($p<0.05$) levels than tertiary-treated samples, whereas tertiary-treated samples had significantly higher DMP ($p<0.05$) and BPA ($p<0.05$) levels than secondary-treated samples. DOP, on the other hand, was higher in secondary-treated samples ($p<0.05$). The levels of DEHP and DEP, on the other hand, did not alter considerably between the two treatments.

### 4.5. Weekly fluctuation of BPA and PAEs concentrations

The levels of all PAEs clearly altered during the course of the weekly sampling period. DEP, DBP, and BBP levels were at their highest in week 4, whereas DMP, DEHP, and DOP levels were at their lowest, according to Tukey's test. DEHP levels were highest in the first week. Daily changes in the residential trash arriving at WTPs could have produced variations in PAEs levels overtime.

## 5. Discussion

In the current study, chromatographic analysis of BPA and six phthalate esters was performed in 250 water samples obtained from 5 wastewater treatment plants (WTPs). The study validated and updated the measurements achieved 5 years ago by a parallel study conducted by Al-Saleh et al. [4]. We reported that both DEHP and DBP were found in all treated wastewater samples. Interestingly, BPA, DMP, and DEHP were higher in tertiary than secondary-treated samples. Comparing between the water plants types (secondary and tertiary), regarding the changes in PAEs and BPA levels, a study carried out by Gani et al. [19], revealed that the WTPs are only able to remove 18% of DEP, DBP, BBP, and DEHP in primary treatments, however tertiary treatment can remove about 93% of the 4 compounds.

Parallel to such findings, Sun et al. [20] encountered difficulty in extracting BPA during the process of treatment. The efficiency of its removal depends, to great extent, on the molecular weight and polarity of such contaminates [21]. BPA is a somewhat hydrophobic chemical that could affect its partitioning behavior and the capability of its removal from the water and soil [22]. Such characteristics could explain the higher levels of BPA in tertiary-treated water samples. Regarding DMP, it has better polarity and easily passes the tertiary treatment due to its short chain length, and smallest molecular weight. DEHP has low solubility under normal resting conditions so, partially appears in the tertiary outflow as demonstrated by Cousins and Mackay [23] and Katibi et al. [24]. In addition, the variation in the technical methods of treatment could influence the efficacy of pollutant elimination [25].

When discussing the findings of the weekly sampling, all PAEs are markedly changed. DEP, DBP, and BBP levels reached their greatest value in week 4, while DMP, DEHP, and DOP levels were lowest. Variations in PAE levels over time may be due to the daily frequent changes in the source of residential trash coming to treatment plants. Comber et al. [26] found that samples analyzed on a monthly basis, the variation in the levels of trace organic pollutants per day/week was parallel, which was due to the cyclic (seasonal) variations (fluctuations) in water- and chemical-use activities at the source, which could affect wastewater composition of contaminants and site features and characteristics, hence the treatment efficiencies.

Concerning the toxicological assessment of toxins, the acceptable level of exposure is determined by the competent authorities, all over the world, based on a wide variety of animal studies [27]. For example, U.S. Environmental Protection Agency [28] announced 0.05 mg/kg/day to be the intake limit for BPA, while the EFSA and the European Commission (EC) have announced a lower TDI value (0.004 mg/kg/day) for the same compound [29]. Based on the EFSA/EC TDI approach, the determined average concentration of BPA may be alarming and associated with a potential risk for humans. Meanwhile, the measured mean concentration of DBP is below the aggregated dietary exposure for average DBP consumption (0.9 µg/kg) as announced by ESFA, 2019 [30].

## 6. Conclusion

In Saudi Arabia, the present study detected phthalate esters and BPA in treated wastewater used for agriculture, regardless of the treatment type utilized (secondary or tertiary). Both PAEs and BPA have proven to cause detrimental reproductive and developmental deficits [31], so the findings should pay attention of the Saudi agriculture, because the long-run irrigation with treated wastewater could lead to soil contamination by PAEs and BPA, reach human and animals via the food chain. Efficient WTPs are needed to extract these chemicals more effectively. Till, now, treated wastewater should be used cautiously for agricultural purposes to ensure healthy food chains free of contamination.

## 7. Conflict of interest

The authors have declared no conflict of interest.

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**Table 1:** Levels of BPA and 6 phthalate esters in treated wastewater samples obtained from 5 Saudi wastewater treatment plants.

| Analytes (ug/L) | Water plants | Number of samples | Mean | SD | Median | Min | Max | >LOD | >LOQ |
|---|---|---|---|---|---|---|---|---|---|
| BPA | Al Hofuf | 50 | 3.835652 | 0.376185 | 3.826475 | 3.20736 | 4.54703 | 8 | 6 |
| | King Saudi University | 50 | 4.177342 | 0.426454 | 4.176776 | 3.46608 | 4.88785 | 35 | 19 |
| | Manfouha | 50 | 3.848691 | 0.057604 | 3.84469 | 3.75556 | 3.94424 | 21 | 15 |
| | Wadi Al-Araj | 50 | 6.771998 | 0.103396 | 6.77483 | 3.58434 | 6.92474 | 30 | 28 |
| | Wadi Hanifah | 50 | 3.604202 | 0.012667 | 3.605335 | 0.36205 | 3.62383 | 19 | 6 |
| | Total | 250 | 4.447577 | 1.204816 | 3.86497 | 0.36205 | 6.92474 | 113 | 74 |
| DEHP | Al Hofuf | 50 | 0.366361 | 0.00264 | 0.365885 | 0.34023 | 0.37075 | 4 | 0 |
| | King Saudi University | 50 | 0.351492 | 0.006816 | 0.35177 | 0.34023 | 0.36189 | 8 | 6 |
| | Manfouha | 50 | 0.429879 | 0.005461 | 0.430095 | 0.41956 | 0.43773 | 4 | 2 |
| | Wadi Al-Araj | 50 | 0.944182 | 0.003324 | 0.944035 | 0.3633 | 0.94856 | 6 | 5 |
| | Wadi Hanifah | 50 | 0.41451 | 0.028861 | 0.41381 | 0.3633 | 0.47469 | 13 | 10 |
| | Total | 250 | 0.501285 | 0.223766 | 0.41381 | 0.34023 | 0.94856 | 35 | 23 |
| DMP | Al Hofuf | 50 | 0.472964 | 0.008274 | 0.472185 | 0.13126 | 0.48689 | 19 | 10 |
| | King Saudi University | 50 | 0.141913 | 0.006789 | 0.142081 | 0.08201 | 0.1529 | 0 | 0 |
| | Manfouha | 50 | 0.086465 | 0.002538 | 0.086545 | 0.08201 | 0.09098 | 2 | 0 |
| | Wadi Al-Araj | 50 | 0.371526 | 0.007949 | 0.370085 | 0.17665 | 0.38628 | 0 | 0 |
| | Wadi Hanifah | 50 | 0.182317 | 0.003885 | 0.182074 | 0.15267 | 0.18893 | 2 | 2 |
| | Total | 250 | 0.268217 | 0.146754 | 0.18213 | 0.08201 | 0.38628 | 23 | 12 |
| DOP | Al Hofuf | 50 | 0.171886 | 0.010664 | 0.169705 | 0.04601 | 0.19249 | 10 | 0 |
| | King Saudi University | 50 | 0.070796 | 0.014813 | 0.070993 | 0.04601 | 0.09856 | 0 | 0 |
| | Manfouha | 50 | 0.570646 | 0.220993 | 0.54899 | 0.12267 | 0.95507 | 1 | 0 |
| | Wadi Al-Araj | 50 | 0.149175 | 0.015079 | 0.14769 | 0.12267 | 0.1758 | 10 | 0 |
| | Wadi Hanifah | 50 | 0.309835 | 0.106924 | 0.313355 | 0.12444 | 0.50675 | 0 | 0 |
| | Total | 250 | 0.254467 | 0.207609 | 0.16969 | 0.04601 | 0.95507 | 21 | 0 |
| DEP | Al Hofuf | 50 | 0.179935 | 0.009252 | 0.180663 | 0.09969 | 0.19511 | 0 | 0 |
| | King Saudi University | 50 | 0.129318 | 0.020216 | 0.13188 | 0.09969 | 0.16181 | 0 | 0 |
| | Manfouha | 50 | 0.176239 | 0.006428 | 0.177745 | 0.13706 | 0.18584 | 0 | 0 |
| | Wadi Al-Araj | 50 | 0.146461 | 0.006439 | 0.14588 | 0.13706 | 0.1582 | 0 | 0 |
| | Wadi Hanifah | 50 | 0.313154 | 0.062936 | 0.320725 | 0.21417 | 0.40375 | 10 | 0 |
| | Total | 250 | 0.189021 | 0.189021 | 0.189021 | 0.09969 | 0.1582 | 10 | 0 |
| DBP | Al Hofuf | 50 | 0.571689 | 0.063708 | 0.557635 | 0.47144 | 0.68637 | 24 | 4 |
| | King Saudi University | 50 | 0.718998 | 0.063252 | 0.723865 | 0.60989 | 0.82083 | 32 | 13 |
| | Manfouha | 50 | 1.496153 | 0.123044 | 1.49766 | 0.71122 | 1.72014 | 30 | 21 |
| | Wadi Al-Araj | 50 | 0.84597 | 0.086706 | 0.837645 | 0.3077 | 0.98931 | 24 | 0 |
| | Wadi Hanifah | 50 | 0.39245 | 0.052635 | 0.39275 | 0.10744 | 0.51571 | 30 | 26 |
| | Total | 250 | 0.805052 | 0.385976 | 0.72076 | 0.10744 | 0.51571 | 140 | 64 |
| BBP | Al Hofuf | 50 | 0.164052 | 0.033104 | 0.167335 | 0.10744 | 0.21795 | | |
| | King Saudi University | 50 | 0.411 | 0.061548 | 0.41096 | 0.30666 | 0.50601 | 10 | 0 |
| | Manfouha | 50 | 0.540498 | 0.122988 | 0.544519 | 0.01778 | 0.73643 | 10 | 0 |
| | Wadi Al-Araj | 50 | 0.122109 | 0.05576 | 0.13103 | 0.01778 | 0.21835 | 0 | 0 |
| | Wadi Hanifah | 50 | 0.704031 | 0.088138 | 0.699205 | 0.55156 | 0.88089 | 21 | 10 |
| | Total | 250 | 0.388338 | 0.23469 | 0.37667 | 0.01778 | 0.21795 | 41 | 10 |

## 10. Authors' contribution

All authors equally contributed to the current study.

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