An-Najah National University

Faculty of Graduate Studies

ASSESSING THE PREVALENCE OF PHARMACEUTICAL RESIDUES IN WADI ZOMAR CATCHMENT AREA IN PALESTINE: RISK ASSESSMENT FOR REUSE AND IMPACT ON HUMAN HEALTH

By

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Dedication

In the name of Allah Almighty, the one to whom gratitude and thanks are acknowledged before anything. Allah has planted the first seed of science passion in me and guided me to the completion of this thesis.

Second of all, I sincerely thank all those who believed in me, pushed me, and helped me through this journey; my parents and siblings, are the unknown soldiers.

For all those who believe in science and human well-being. For all of them, I dedicate this and every work.

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Declaration

I, the undersigned, declare that I submitted the thesis entitled:

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I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name Ala'a Jaradat

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ASSESSING THE PREVALENCE OF PHARMACEUTICAL RESIDUES IN WADI ZOMAR CATCHMENT AREA IN PALESTINE: RISK ASSESSMENT FOR REUSE AND IMPACT ON HUMAN HEALTH

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Abstract

The occurrence of various classes of antibiotics and pharmaceuticals (PhCs) in the environment and their contribution to Antimicrobial resistance (AMR) development is questionable. AMR is recognized as a major health threat. Discharges from wastewater treatment plant (WWTP) is considered to be the major source contributing to the vast bulk of different pharmaceuticals in the environment. The researcher intends to investigate the prevalence of pharmaceutical residues in diverse aquatic matrices with more attention to the removal capacity of West Nablus WWTP with respect to the detected pharmaceuticals. *Method*: 2 raw wastewater, 2 treated wastewater, and 2 surface wastewater run-offs were collected in two grab sampling campaigns. An additional ground water sample was collected using a Passive Organic Chemical Integrative Sampler (POCIS). All samples were analyzed using LC-MS/MS. The Risk Quotient (RQ) was used for Measured Environmental Concentration (MEC) of detected antibiotics against Predicted No-Effect Concentration ((PNEC) to evaluate the risk for antibiotic resistance development according to the detected antibiotics residues. Sulfamethoxazole, Trimethoprim, Diclofenac, and Carbamazepine were the most frequently detected in all water samples. West Nablus WWTP delivered a significant removal efficiency in both campaigns. However, it was noticed that there was a significant spatial difference between the WWTP effluent discharge point and the Anabta-Zomar point of sampling directly after the rainy season. Ofloxacin residual concentration in immediate WWTP effluent discharges and surface run-offs along the sampling point is found to pose risk for AMR development in the environment. Groundwater is found to be polluted with Carbamazepine, Diclofenac, Ciprofloxacin, and Sulfamethoxazole. The local status indicates the need for further in-depth investigation regarding the risk of antibiotics to the environment and its role in the emergence of AMR concerning the detected antibiotics. Additional wastewater

treatment methodologies are needed for better removal yield. Groundwater pollution requires urgent ecotoxicological studies for both human and animal health and environmental life forms.

Keywords: Antibiotics; Antimicrobial resistance; Bacteria; Environment; Health; Pharmaceuticals; Wastewater; WWTP.

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Chapter one

Introduction and Literature Review

A healthy environment is as healthy as its smallest components can get to sustain its normal function. This is jeopardized as pharmaceutical (PhCs) pollutants of different sources are frequently found with different toxicities impacting various life forms(1). The use of different classes of therapeutics is indispensable for human and veterinary purposes, by which they are continuously being introduced into the environment(2–4).

Antibiotics usage is intended to combat infectious conditions either as a treatment or as a prophylaxis, both in human and veterinary fields. Upon their administration, they undergo several metabolic processes by which they are broken down into active and inactive metabolites. None the less, not all administered dose is metabolized and a portion of the active parent drug remains intact. The excretion is limited to the urinary and/or biliary tracts (5).

The veterinary field relies on the use of antibiotics extensively as 73% of the global production of antibiotics is intended for animals raised for food as Animal Growth Promoters (AGPs)(6). Even though the excretion of antibiotics in animals is species-dependent, their manure is found to harbor several classes of antibiotics and other pharmaceutical compounds (3,7).

This is of concern for two reasons. The first reason is the implication of antibiotics use in animals and the emergence of antimicrobial resistance in zoonotic pathogens. The second reason is that manure is regarded as a natural fertilizer for crops and hence the dissemination of several pharmaceutical pollutants into the environment is a plausible possibility(8–10).

The toxic effects PhCs exert on environmental life forms have been recognized after the near extinction of vultures in Pakistan in the time period of (2000-2003). The implicated PhC was found to be diclofenac which led to fatal renal disease. This PhC gained entry to the food chain by the administration to prey vultures feed (4).

After this, the environment has been heavily investigated for the presence of different PhCs and possible access sources as follows. Pharmaceuticals can gain access to the

environment (soil and water) in several anthropogenic routes (figure 1.1): (a) by the direct disposal of pharmaceutical wastewater into receiving environment (from manufacturing facilities or any health care setting), (b) the use of pharmaceuticals in aqua culture, (c) animal PhCs-rich manure utilized for crops production and surface run-off with rain events, (d) treated municipal and industrial wastewater discharged into surface water, and (e) the use in agriculture(3,7,11–13).

Wastewater Treatment Plants (WWTP) have been recognized as the main source of antibiotics and other PhCs introduction into the aquatic environment (11,12,14). WWTPs are typically designed to remove organic and some in-organic pollutants before the effluent is discharged into receiving body. Pharmaceuticals of any class aren't removed in the process and their presence and effect in treated wastewater have been noticed. Where surface water is the end recipient for treated wastewater effluents. It's evident that trace levels (nanogram/Liter) of Endocrine Disturbing Substances (EDS) in the receiving aquatic mediums are sufficient for devastating biological alterations in fish (2,15).

Figure 1.1

The introduction of antibiotics into the environment and the direct and in-direct human exposure to antibiotics

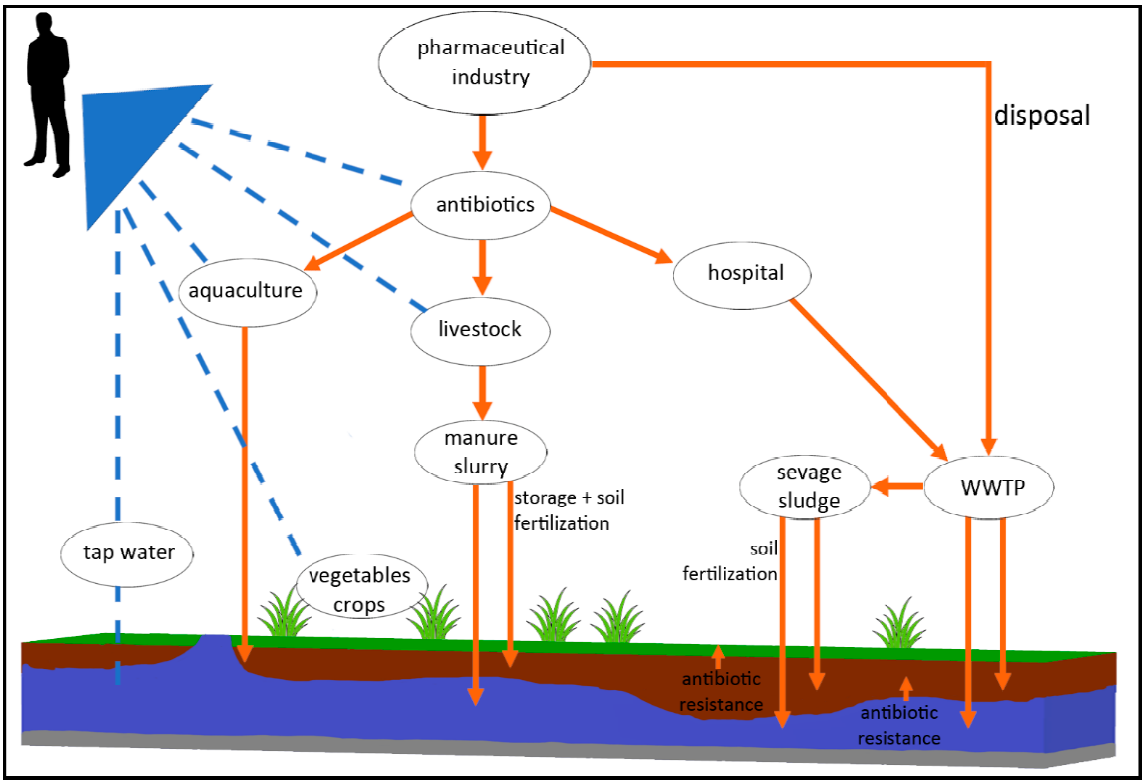


Figure 1.1 displays how antibiotics have gained special attention after their detection in nature. Their delivery into the environment is via different anthropogenic routes. Hospital wastewater, animal manure, aqua culture and municipal wastewater. The cycle continues for human indirect exposure through contaminated water, crops and food-animals. Figure adopted from *Serwecińska et al*(16).

Several studies reported antibiotics detection in aquatic compartments implying the in-ability of the WWTP to remove such PhCs before effluents are discharged. Tetracycline was detected at both the wastewater treatment plant (WWTP) influent and effluent in Spain with a residual concentration of 0.067 µg/L and 0.018 µg/L, respectively (17). Ciprofloxacin was detected in the raw influent of WWTP in Baghdad with a residual of 1.344 µg/L(18).

The awareness of characteristics promoting the stability of PhCs in various environmental compartments aids in the quest to establish mitigation measures. Those characteristics helped PhCs to be considered recalcitrant and resist degradability in water and soil. They range from physiochemical properties (solubility and pH, net charge, long half-life, carbon content, etc.…), to resistance to biodegradation, resistant to solar degradation, and sorption and de-sorption ability.

Sulfonamides, for instance, are an essential class of antibiotics used in both the human and animal medical fields. They were found to be labile for solar-degradation when exposed to direct sunlight. Furthermore, the speed of degradation is compound specific. Sulfamethoxazole was found to be, though to a lesser extent, sensitive for photo-degradation when irradiated with Ultra-Violet A/ Ultra-violet B (UVA/UVB) and direct sun exposure for 7 days (19). While *J.K. Challis et* al predicted the photodegradation of Sulfapyridine in water to be around 2.6 hours (20).

Carbamazepine (CZB) physiochemical properties of hydrophobicity and net-charge of zero have made it to be resistant to solubility in aquatic mediums rendering it a mobile agent. Carbamazepine and Diclofenac have been recognized among the most frequently reported pharmaceuticals on a global scale (21).

Crops cultivated in soil- or irrigated with reclaimed water or contaminated surface water are found to uptake such PhCs from the soil. In a study investigating the occurrence of PhCs in Nakivubo wetlands in Kampala, Uganda, trimethoprim was found to have accumulated in the soil of wetlands and yam roots(22). Moreover, the ecotoxicity is concentration dependent. For example, CZB toxicity in inhibiting the growth rate of *P. subcapitata* is observed at a concentration of 10.4 mg/L while 6 mg/L of Diclofenac is sufficient for the same effect (23).

The integration between factors favoring the accumulation of pharmaceutical pollutants in soil (i.e. chronic introduction into surface water with resistance to degradability and adsorption to soil particles) aids in the seep and detection of such PhCs in groundwater(24).

Drinking water is also found polluted as well. In filtered tap water in China, the average total antibiotic concentration was found to be 0.182 µg/L(25). 0.096 µg/L of Sulfamethoxazole was detected in the effluent of a WWTP in Jordan(12). *Dalahmeh et al.* reported that Trimethoprim and Sulfamethoxazole were the most frequent antibiotics detected in various water samples collected from different sources in Kampala – Uganda(22).

In addition to the environmental aspects(1,26), human health is in question. A study conducted in China estimating sources contributing to the un-intentional exposure of antibiotics through food and water, the main source was found to be food products derived from plants. In addition, preschool children had the highest daily intake rate of antibiotics from drinking water and food (310 ng/kg-bodyweight/day) (27).

The frequent detection of antibiotics in water and soil mediums opens new arrays for research on its implication in the emergence of antimicrobial resistance (AMR). Water provides a rather supporting medium where resistance can get allocated easily. In addition, it supports its persistence and wide spread detection(28).

It has been demonstrated that antibiotics detection in effluents of WWTPs is associated with the detection of resistant bacteria as well(29,30). Concerning the constantly nutrient-rich medium of wastewater with the presence of antibiotics and bacterial

populations as well, *L. Rizzo et al.* in his review, regarded urban WWTP as hot spots where resistance genes and resistance bacteria can easily disseminate to near-by environment. Resistance traits can be exchanged between bacterial colonies.(14).

Antimicrobial resistance (AMR) is considered an urgent health crisis requiring prompt action to tackle. In 2019, the AMR bacteria-associated death toll reached 4.95 million on a global scale (31). This death toll is estimated to reach 10 million by the year 2050 unless an effective response is initiated. The economic impact is extravagated as well, with an expected 100 trillion dollars loss and a gradual decrease in global domestic production by 2 - 3.5% (32).

The etiology underlying the development of AMR is rather ubiquitous. It can be as simple as a natural intrinsic phenomenon(33), or evolutionary response mechanisms bacteria develop due to selection pressure when co-existence with antibacterial levels (34,35) or it can be picked-up from the environment through horizontal gene transfer (36,37). *Pereira et al* in their systemic review regarding the toxicity and environmental risk assessment (ERA) of selected PhCs in different aquatic compartments, noted that antibiotics risk quotients alert for toxicity effects on the environment (1).

To have a clearer picture regarding AMR development in any setting, one needs to understand both parties apart and combined – bacterial spp with/without AMR genes and antibiotics. Bacteria are diverse microorganisms and are considered integral normal inhabitants of any biosystem. They are resilient to function in rather complicated conditions naturally(38). Whereas antibiotics are merely chemical compounds with a variety of pharmacological and physiochemical characteristics that help them achieve their intended function: infectious disease treatment. Nevertheless, the same properties have aided in their persistence in many environmental compartments and their identification (39).

If low levels of antibiotics and certain bacterial spp combine, then the susceptible bacteria will die while the resistant strain will dominate. This is the definition of selection pressure and the evolution of antimicrobial resistance. This level is referred to as Minimal Selective Concentration (MSC)(37,40).

Interestingly, it's noticed that selection develops in concentrations well below the Minimally Inhibitory Concentration (MIC) thresholds proposed for clinically relevant bacteria by several folds (MIC: the lowest level of antimicrobial required to inhibit the growth of microorganisms in-vitro aided by the naked eye) (41,42). *Gullberg et al* reported that the MSC value for ciprofloxacin is 100 pg/L ≈$1/10^{th}$ MIC. In addition, AMR mutation is developed within susceptible bacteria in the presence of MSC despite the presence of already resistant bacteria (41). While the process may not be explained as intentional development rather than a survival and adaptation mechanism for microorganisms' use, its consequences are prevalent in the detection of AMR genes in the environment and the emergence of "super-bugs"(36,43).

On a higher level, environmental risk assessment adopted by the European Medicines Agency (EMA) is used to set-up threshold values for any drug residual in environmental compartments as follows: using the PNEC and Predicted Environmental Concentration (PEC: predicted level of antimicrobial to reach the environment); in order to evaluate their potential risk on the environment. A Risk Quotient (RQ) is calculated by obtaining the ratio of Measured Environmental Concentration (MEC) or PEC to PNEC for each pharmaceutical compound of question. If the RQ is > 1 then a potential impact is predicted on different trophic levels, and this calls for further studies determining what the effect shall be. Whereas if the RQ is < 1, then no risk is expected (44).

In terms of the dual detection of residual concentrations of antibiotics and resistant bacteria in the environment, efforts are done to clarify the point where a certain concentration of an antibiotic paves the way for resistance development. Scientists *Bengtsson-Palme and Larsson et al.*developed Predicted No-Effect Concentrations (PNEC) derived from the MIC values established in the European Committee on Antibiotic Susceptibility Testing (EUCAST) for the majority of frequently detected antibiotics in the environment (PNEC –MIC: a level of which below no effect on the clinically relevant bacteria is observed – according to the MIC values). PNEC-MIC took into consideration the effects of antibiotics on clinically relevant bacteria (45).

On the other hand, the Environmental Predicted No-Effect Concentrations (PNEC-ENV) developed by *Le Page et al*(46) studied the effects of antibiotics on representative environmental bacteria (Cyanobacteria, *V. fischeri* and *P. putida*) (46).

The residual concentration detected in the environment for antibiotics is then compared to the PNEC value to estimate the RQ value. If RQ is >1, then this antibiotic is expected to exert selection pressure against selected species and promote resistance evolution(45,46).

The AMR Industry Alliance is a group of several pharmaceuticals, advance research, and diagnostic companies of the private sector collated to curb AMR. The alliance advocated the lowest value of PNEC-MIC and PNEC-ENV thresholds as antibiotics target markers for manufacturing companies to adhere to when considering pharmaceutical wastewater discharge into the environment. Those values are updated regularly. In addition, the alliance recommended a default threshold of 0.05 μg/L for antibiotics when no PNEC value is available(47).

Several antibiotics are on the EU watch list of substances within the European Water Framework Directive on a union-wide monitoring system for high-quality data gathering. The watch list is established for substances whose residuals are potentially found to pose a significant risk to, or via, the aquatic environment and is updated every 2 years. Currently, of those substances the antibiotics: Trimethoprim, Sulfamethoxazole, Ofloxacin, and Clindamycin(48).

Up to this point, ground water, surface water, wastewater, vegetables, and animal manure are all found to harbor residual concentrations of antimicrobials (18,49). This brings to mind several questions. First: what level of risk do those trace residues pose on human health? Second: the acute versus chronic exposure effects to the emerging new contaminant? Third: when should we include screening for antimicrobials as "pollutants" as part of regulating water use? And the clear need to implement advance wastewater management technologies.

In the West Bank, the presence of several classes of antibiotics and herbicides was investigated in off-grid, household greywater reuse systems in farms where the

reclaimed water is intended to be used for crop irrigation. Several antibiotics were recovered from domestic sewage, filtered sewage, and pond water with varying concentrations and frequencies. Pipemidic acid, Azithromycin, and Oxolinic acid were the most frequent antimicrobials among the influents while only Oxolinic acid was the most frequent in the effluent samples(50). So far to the authors' knowledge, published work regarding the detection of antibiotics in different aquatic compartments locally is absent.

Palestinian hydrological sources are scarce. Their exploitation is rather restricted which imposes further pressure on the quality and proper utilization of accessible natural resources (51). This highlights the importance of aquatic management practices especially groundwater (52). Reclaimed wastewater is proposed as an alternative for agricultural purposes to elevate the demand for groundwater intended for agricultural consumption.

AMR is regarded as a major public health issue and the local status indicates the presence of high resistance to antibiotics including last resort antibiotics: colistin, from both clinical human samples and animal samples (9,53–55). Given that there are no local studies regarding the role of aquatic environments in disseminating resistance and the urgent need to tackle AMR, we looked for the contribution of municipal and industrial sewage in occurrences of antibiotics in several water types (raw sewage, treated wastewater, treated surface wastewater and ground water). The West-Nablus WWTP is used here as a case study.

Aim and Objectives

We aim to investigate the occurrence of antibiotics and other pharmaceuticals (PhCs) in raw, treated, surface wastewater and ground water in west Nablus, North Palestine.

Specific objectives:

- To evaluate the removal efficiency of west Nablus WWTP for the detected PhCs if present.

- To test for the spatial difference in concentrations of detected compounds in collected samples.
- To link between dispensary quantities of antibiotics from hospitals in the study area and the identification of antibiotics in wastewater.
- To evaluate the potential health effect of measured environmental concentrations of antibiotics and the development of AMR according to the data in the literature.

Chapter Two

Materials and Methods

2.1 Study area

The study area covered the catchment area of west Nablus in the West Bank of Palestine. All the catchment area untreated sewerage is drained down to West Nablus WWTP. The WWTP inlet was taken as the first sampling location (raw wastewater) just before entering the WWTP. The finished treated wastewater (WW) was considered the second WW sampling site which is directly discharged into Wadi Zomar. Treated WW transports down Wadi Zomar through Anabta village where the third sample was collected at Anabta at a specific location for both sampling campaigns.

2.1.1 West Nablus WWTP

Nablus city is located north of Palestine and 681 m above sea level. The WWTP is located west of Nablus and is 305 m above sea level. It receives sewage (domestic, industrial, and pharmaceutical) from west Nablus catchment area alongside 5 surrounding villages (Zawata, Beit Eba, Dair Sharaf, Beit Wazan, and Qusin), with approximate inhabitants of 120,00. The plant is in service since 2015 and operates as a secondary treatment facility. It uses activated sludge with mechanical treatment, biological treatment, and sludge treatment steps with gasutilization.

West Nablus WWTP was designed to receive an estimated flow of raw sewage water of about 14,000 m^3/day. In 2022, it received an average of 12,000 m^3/day. According to West Nablus WWTP annual report of the year 2022, Biological Oxygen Demand (BOD) and Total Suspended Solids (TSS) average values in the inlet WW were 535 mg/L and 440 mg/L, respectively. WWTP performance capacity for the removal of BOD and TSS was 98% for both parameters (56). The treated wastewater is continuously being discharged into the environment of Wadi Zomar. This wadi passes through Anabta village, Tulkarem city (63 m above sea level), and finally into the Mediterranean Sea.

Figure 2.1

Location of (a) West- Nablus WWTP and sampling points with surrounding communities in respect to Wadi Zomar. (b) Palestine/ West Bank water supply map

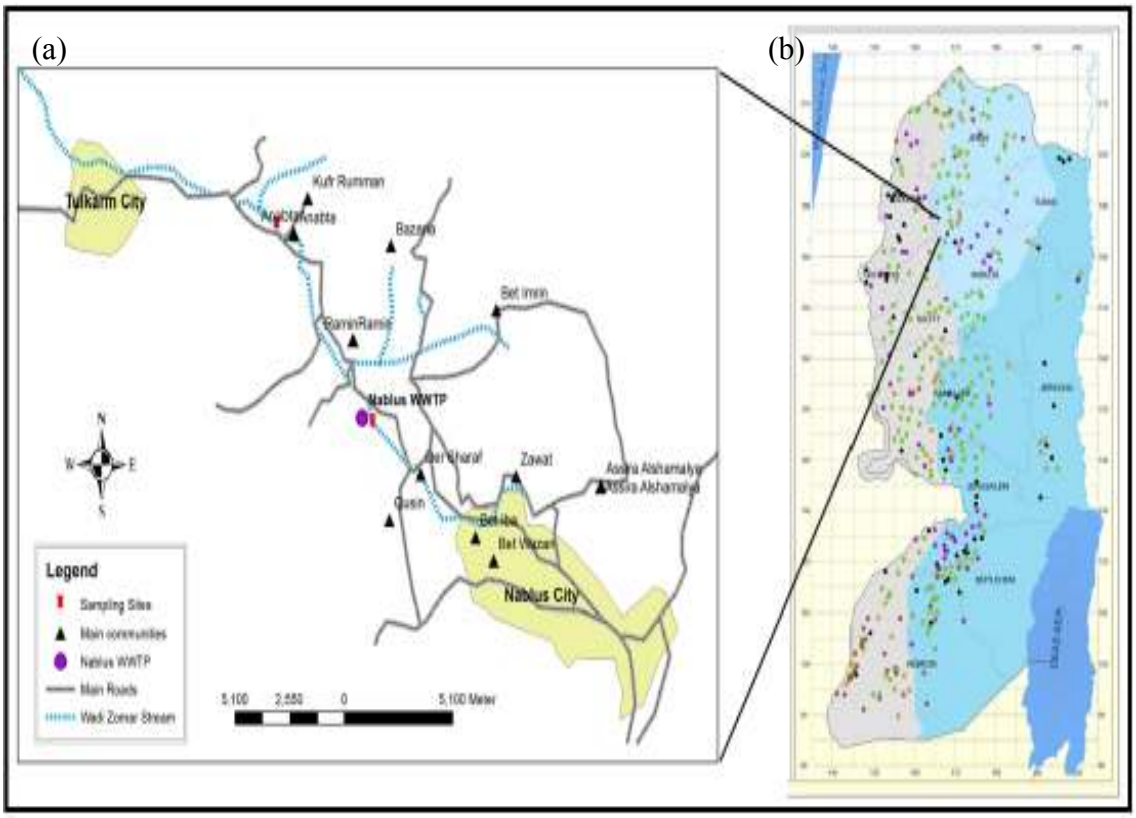


Figure 2.1 (a) displays the geographical locations of sampling points (red pin) at the inlet of west Nablus WWTP (purple circle) and outlet point and at Wadi Zomar-Anabta (red pin). Wastewater at Wadi Zomar has many tributaries (from Bet Imren, Bazaria and Kufr Rumman) along its natural path as displayed here. (b) Palestine/west bank water supply map adopted from Palestine Water Authority website.

2.1.2 Wadi Zomar

The importance of Wadi Zomar originally comes from the fact that it's the largest treated wastewater discharge in the northern part of the west bank. It's 55 Km from the upper part of Alexander River in northern Palestine originating from the mountains of the West Bank and reaching the coastal plane. The investigated area of Wadi Zomar lies between West Nablus WWTP and Anabta village which compromise 5 Km.

Where Wadi Zomar passes through the village and is surrounded by several groundwater wells. Most of these wells are municipal wells and some are agricultural wells.

Figure 2.2

Vulnerability map of the studied area displaying the geological and hydrogeological structures

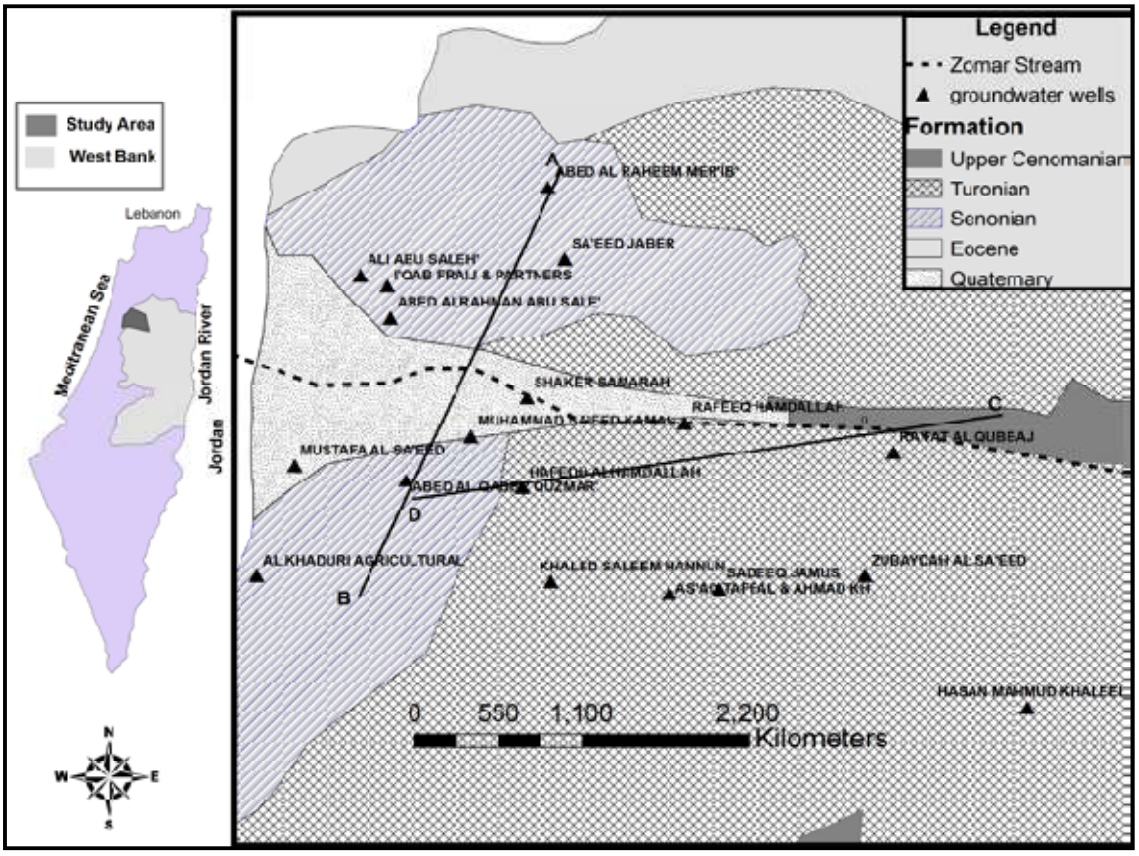


Figure 2.2 explains the geological structure around and underneath Wadi Zomar. It has diverse geological structures that permits the seep of different pollutants to layers below earth surface where wells are dugged normally. Vulnerability map Adopted from Saed Khayat et al.: Mechanisms of Groundwater Pollutants Transport in Tulkarm Area / Palestine, Resources and Environment 2012, 2(6): 281-290.

The geological and hydrogeological structures of the studied area highly influence the infiltration of different pollutants into the sediment bed of Wadi Zomar. The main aquifer for Palestinians in the northern region of the west bank comes from the western and north-eastern water basins. Most of the groundwater wells are dugged in the shallow layer of the uppermost Jerusalem-Turonian aquifer which has characteristics that facilitate the movement of pollutants from the top layer (57–59).

2.2 Ethical approval

This study was reviewed and approved by the An-Najah National University (ANNU), Institutional Review Board (IRB) (Appendix A). Designated facilities were informed of the scope and intent of the research before the initiation of samples collection. Approvals from hospitals, Nablus municipality and Anabta municipality were granted prior to antibiotics dispensary data collection (Annexes B-G).

2.3 Antibiotics dispensary data collection

To have a baseline data regarding the expected human excretion of both quantity and class of antibiotics in the study area, we've requested dispensary data regarding antibiotic consumption from all hospitals located in West Nablus in the period of 1/1/2021 – 28/2/2022. Out of the 7 hospitals residing in west Nablus, 5 hospitals (2 governmental and 3 private hospitals) agreed to provide us with the required data.

All hospitals' domestic sewerage is piped into the same system; with no previous treatment where all raw wastewater with PhCs residues drained directly to west Nablus WWTP. Medical waste (biomedical waste containing hazardous materials to human and environment) from hospitals are subjected to special waste disposal practices. Hospitals domestic sewerage isn't included in such practices and the produced wastewater isn't tested for pharmaceutical residues (Personal communication with Mr. Yousef Jaffal, West Nablus WWTP Chief Director and Eng. Suha Kharraz, Environmental Control Unit Chief Director at Nablus Municipality). Approvals from Nablus municipality, Anabta municipality, and all designated hospitals are supplemented in annexes (B-G).

A plant for veterinary PhCs is located in west Nablus study area. Their industrial wastewater isn't disposed down the sewerage systems as they developed an internal protocol that eliminate any disposal alongside west Nablus domestic sewerage (Personal communication with Nivin Ratrot, Dana Pharmaceuticals Quality department Chief Director).

2.4 Sample collection, preparation, and extraction

2.4.1 Water Samples collection

Sampling campaigns were divided into 2 grab sample sets for wastewater and one Passive Organic Chemical Integrative Sampler (POCIS) (Environmental Sampling Technologies, EST Inc, St. Joseph, MO, USA) for groundwater.

Three grab samples per set were collected from raw (WWTP inlet), treated (WWTP outlet), and surface wastewater (from Wadi Zomar at Anabta collection point) in early April/2022 and another set in September/2022 with a total of 6 grab samples. Grab samples were collected in 1L amber glass bottles that were previously rinsed with LC-grade Methanol (Sigma Aldrich, St. Louis, MO, USA), then washed with double deionized water (Chromasolva^{TM} Plus, for HPLC-gradient, obtained from Honeywell Riedel-de Haen, M, USA). Samples were immediately stored on ice bags and refrigerated at 4-8 ˚c until shipment to analyzing laboratory (Royal Scientific Society, Amman, Jordan) within 48hrs of collection.

An additional 2 grab samples were collected from Wadi Zomar at the Anabta sampling point in each campaign, stored on ice bags, and tested for wastewater quality parameters at West Nablus WWTP Lab. Those parameters include Chemical Organic Demand (COD), Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), Conductivity, and pH).

The POCISs were deployed in early April/2022 for 28 days. Two POCIS with Hydrophilic- Lipophilic Balanced (HLB) sorbent membranes were used (Waters Corporation, Milford, MA). Two sets were used to avoid any misleading information regarding temporal variations of measured PhCs in groundwater samples and the measurements from both sets were averaged. The POCIS were constantly submerged at Anabta's municipal groundwater reservoir after water is pumped from the groundwater wells. During installation, caution was taken to avoid heavy water movement (i.e. the pump) near the POCIS. On the 28th day, the POCIS were removed from the ground water tank, wrapped inaluminum foil, kept in an airtight, clean plastic bag, and preserved at 4◦c during transporting and shipping to analyzing laboratory (Royal Scientific Society, Amman, Jordan).

Figure 2.3

The journey of the study, Wadi Zomar at Anabta sampling point, the grab samples of both campaigns, the deployment of the POCIS, the WWTP sampling points and shipment of the grab samples

2.4.2 Chemicals

All chemicals including all reference materials and labeled standards for antimicrobials and PhCs are obtained from Sigma–Aldrich (St. Louis, MO, USA). A list of tested antibiotics and other pharmaceuticals is detailed in Appendix I. Solvents used in sample preparation were of high-grade purity (OPTIMA, Fisher Scientific, St. Louis, MO, USA).

2.4.3 Sample preparation and extraction

The extraction process was implemented according to the procedure provided by Water Sciences Laboratory at the University of Nebraska–Lincoln (WSL/UNL) in the United States (USA) and others (60,61) described as follows.

Grab samples were first decanted to remove all suspended particles and then filtered through the vacuum filtration unit using a 0.45µl glass fiber filter. Then the filtered sample is passed through the cartridges of polymeric Hydrophilic-Lipophilic Balanced

(HLB) Oasis 6CC (200mg) using a vacuum manifold system. The Cartridges were first connected to a solid-phase extraction (SPE) manifold and vacuum pump and preconditioned by passing 6mL of acetone and then 6mL of methanol respectively, followed by passing 6mL of distilled deionized water ($DDIH_2O$). The flow rate of samples through the SPE cartridge was set at 10mL/min or less. The cartridges were then rinsed with $DDIH_2O$ once the extraction process has completed. Drying was done using room air with continued suctioning for no less than 5min. All cartridges are then removed, labeled, and stored in a clean bag at (-20◦c)(61).

POCIS were disassembled once arrived at the analyzing lab and the sorbent was transferred into glass gravity-flow chromatography columns. Chemical residues were recovered from the sorbent by organic solvent elution. Methanol was used to recover analytes from the pharmaceutical POCIS. The extracts were reduced in volume by rotary evaporation and under a gentle stream of nitrogen, then filtered through a glass-fiber filter, solvent exchange was used as necessary, and sealed in amber ampoules under nitrogen until further analysis(60).

2.4.4 Analytical methods

The cartridges were eluted using 10mL of high-purity methanol into a disposable glass culture tube. The volume of the eluent then was reduced to 4mL under nitrogen at 40◦c. Subsequently, it was transferred to auto-sampler vials where the inserts were silane-treated(60,61).

The separation of compounds was done using the AB-Sciex 5500 Qtrap (LC-MS-MS) equipped with Luna Omega polar C18 embedded column (100 mm*3.0 mm, particle size of 3µm). Gradient elution mode was considered in the following order: eluent A is 5mM ammonium formate and 0.1% formic acid, and eluent B is Acetonitrile and 0.1% formic acid. The initial mobile phase conditions started with 98:2 A/B for 1min then a linear gradient pattern was used to reach a ratio of 70:30 A/B at 3min, then the ratios were changed to reach 50:50 A/B at 6min. Before returning to the initial conditions, the last ratio was maintained for 10min. The flow rate was set at 0.6mL/min and the injection volume of 5 µL(60,61).

Multiple reaction monitoring (MRM) was conducted with AB-Sciex 5500 Qtrap equipped with an electrospray ionization interface, using the positive-ion mode (Santa

Clara, CA, USA). Instrument control, data acquisition, and quantitation were run using analyst software. Setting the drying gas temperature at 350◦C, the capillary voltage of 4.0kV, drying gas flow of 12L/m, and the nebulizer pressure of 40 psi(60,61).

Of the 60 PhCs tested, 14 compounds were detected in different samples. Their physiochemical properties (molecular weight g/mol, acid dissociation constant (pKa), Log of the octanol/water partition coefficient (Log K_{ow}), water solubility, Chemical Abstracts Services (CAS) number, and chemical formula) are displayed in Table 3.3.

2.5 Statistical Analysis

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS version 22) to test for the significance of spatial differences between each point of sample collection with respect to seasonal differences as well. Descriptive statistics were reported in tables and figures. Wilcoxon Signed rank test was used to test the spatial differences between samples of the same campaign. The significance level (p-value) was set at 0.05. *Note:* The single detection of a compound among all collected samples in both campaigns is disregarded and excluded in data analysis where further validation is required.

Chapter Three

Results

3.1 West Nablus WWTP performance

According to wastewater quality parameters of West Nablus WWTP, during sampling, the plant showed high efficiency in the removal of BOD_5, COD, and TSS at a rate of 97 %, 94.5 %, and 97% respectively (Table 3.1). All the parameters comply with the top requirements of the Palestinian mandatory standards for wastewater efficient treatment before effluent discharge into the environment (62). Table 3.1 also includes results of the same wastewater quality parameters of the 2 grab samples collected at the Anabta-Zomar point of sampling of both campaigns.

Table 3.1

COD, BOD & TSS parameters of collected wastewater samples obtained from West Nablus WWTP

Test	Unit	First sampling campaign 11th April			Second sampling campaign 19th Sep.		
		WWTP Inlet	WWTP Outlet	Anabta surface WW	WWTP Inlet	WWTP Outlet	Anabta surface WW
COD	mg/l	687	38	78.4	1094	60	157
pH	*	7.76	7.69	NA[1]	7.8	7.59	8.11
Conductivity	µS/cm	1438	1360	NA	1730	1717	1724
TSS	mg/l	118	0	16	504	24	50
BOD_5	mg/l	543	12	11.4	461	12	22

[1]: not available

3.2 Grab Samples and Removal Efficiency

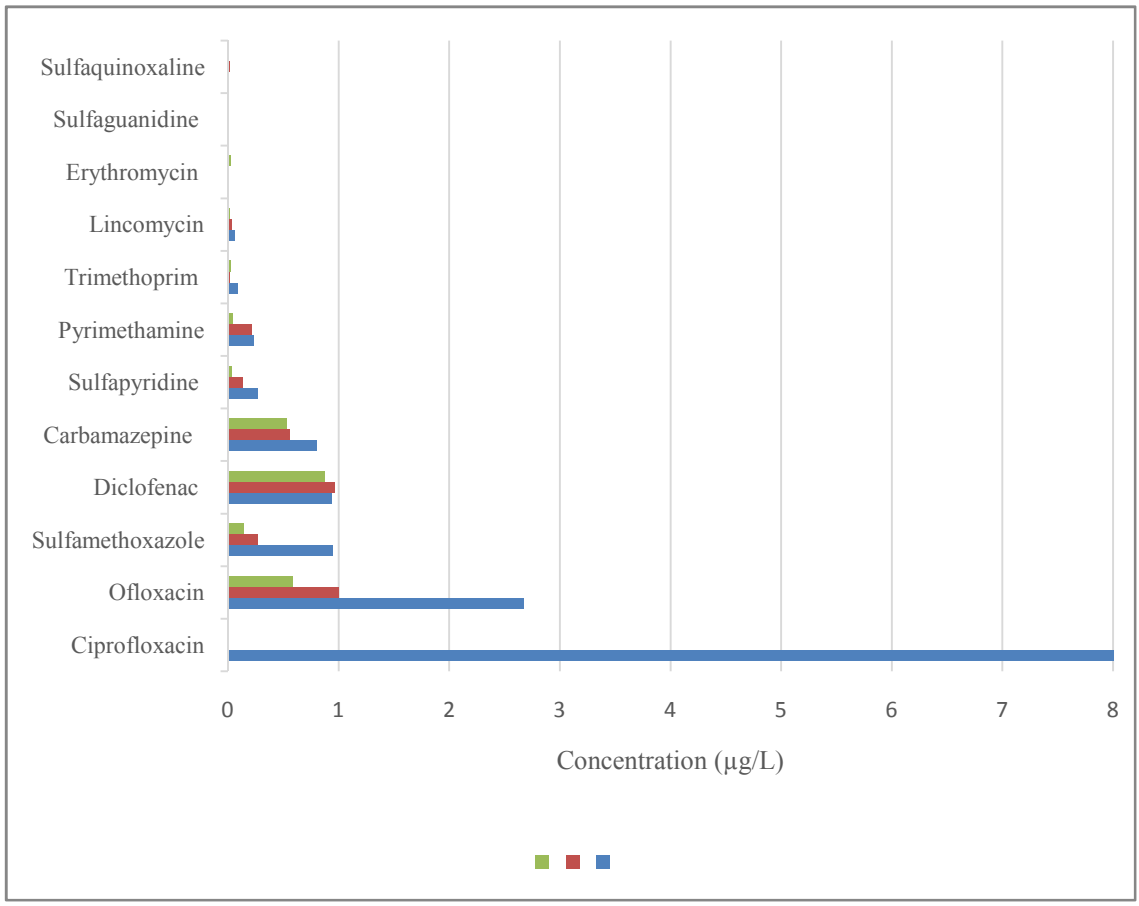
In general, the results of the grab sampling campaigns showed consistency for the majority of detected PhCs and some heterogeneity in some others. In total, 14 PhCs were detected from different classes. Those can be described as follows.

First grab sampling campaign results

Figure 3.1 displays the findings of the first garb sampling campaign, which was undertaken in April 2022. A set of 10 PhCs were found in total for all sampling locations. Of which, 9 PhCs were detected in the WWTP inlet, 10 PhCs were found in the WWTP outlet, and both sampling locations shared 8 PhCs out of 10.

Figure 3.1

Summary of different PhCs detected (µg/L) in the first sampling campaign of April/2022



In comparison to the concentrations of the 10 PhCs detected in the WWTP outlet sample and 9 PhCs WWTP inlet sample, the removal efficiencies of West Nablus WWTP per PhC compound detected are presented in Figure 3.4.

There was a significant difference between the inlet and outlet samples results of the first campaign (Wilcoxon signed rank test, p = 0.025), which in turn reflects the efficiency of west Nablus WWTP in average removal of detected PhCs. Whereas West Nablus WWTP displayed different efficiencies which can be summarized as follow. Complete removal was seen only for CIP 100%, followed by ~80% for Trimethoprim (TMP), 72 % for SXM, 62.4 % for OFX, 50 % for Sulfapyridine (SP), 36.2 % for Lincomycin (LCM), 31 % for CBZ, 5.6% for Pyrimethamine (PYR), and negative removal of -2.7 % for DIC.

Interestingly, West Nablus WWTP treatment revealed the presence of trace amounts of Sulfaguanidine (SGD) and Sulfaquinoxaline (SQX) in the outlet sample where they

were absent in the inlet sample. In addition, Diclofenac residual level increased slightly to reach (0.961 µg/L) in the outlet sample.

A significant spatial difference was found between the concentrations of the PhCs in the WWTP effluent sample and the concentrations of detected PhCs in the wastewater sample in Wadi Zomar at the Anabta sampling location (Wilcoxon signed rank test, $p = 0.016$). It also detected the presence of Erythromycin (E) in trace concentration (0.022 µg/L) which was absent from the previous two grab samples. This in turn suggests the introduction of new raw wastewater prior to this sampling location. SGD wasn't detected in this sample.

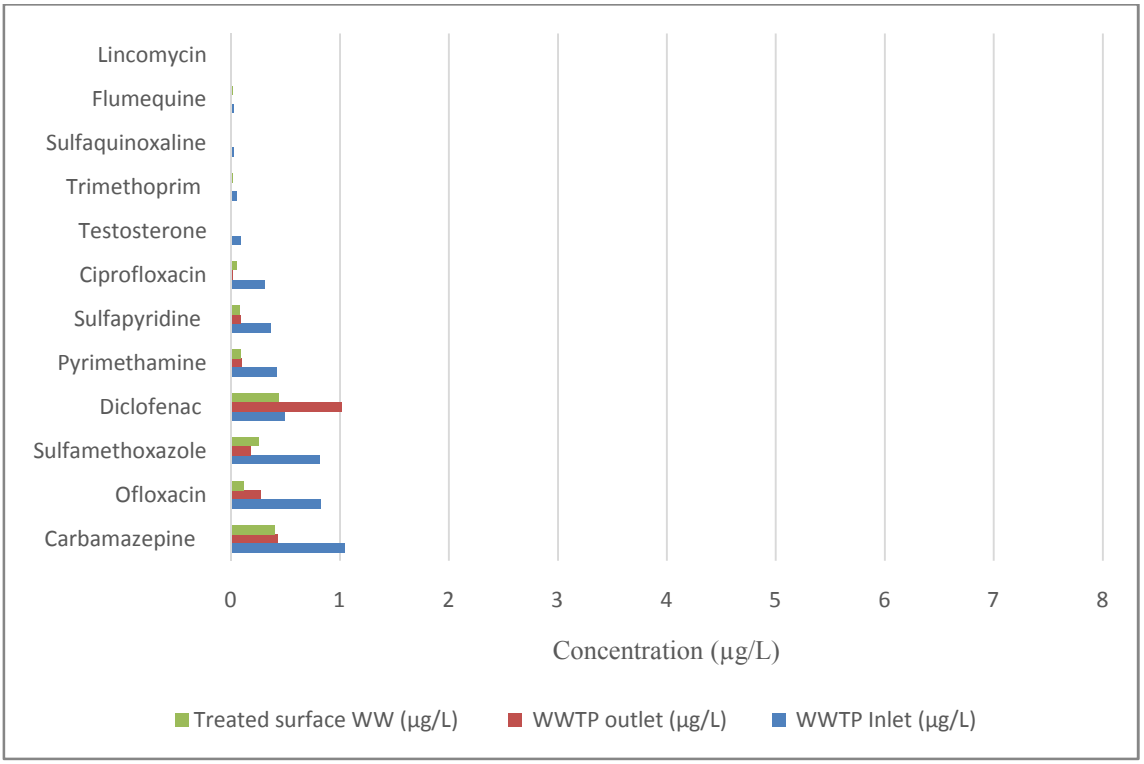
Second sampling campaign results

The results of the second grab sampling campaign done in September/2022 are presented in Figure 7. A total of 11 PhCs are detected in all autumn samples for all sampling locations. 11 PhCs are detected in the WWTP inlet sample, 8 PhCs are detected in the WWTP outlet sample and 9 PhCs were detected in the surface wastewater in Wadi Zomar at Anabta point of sampling. 7 PhCs out of 11 are in common between all samples.

There was a significant difference in the concentrations of detected PhCs between the WWTP inlet and outlet autumn samples (Wilcoxon Signed rank test, $p = 0.033$). The removal efficiency of west Nablus WWTP regarding the detected PhCs in the outlet sample of the second campaign in comparison to the inlet results is in Figure 3.4.

Figure 3.2

Summary of different PhCs (µg/L) detected in second sampling campaign among wastewater samples

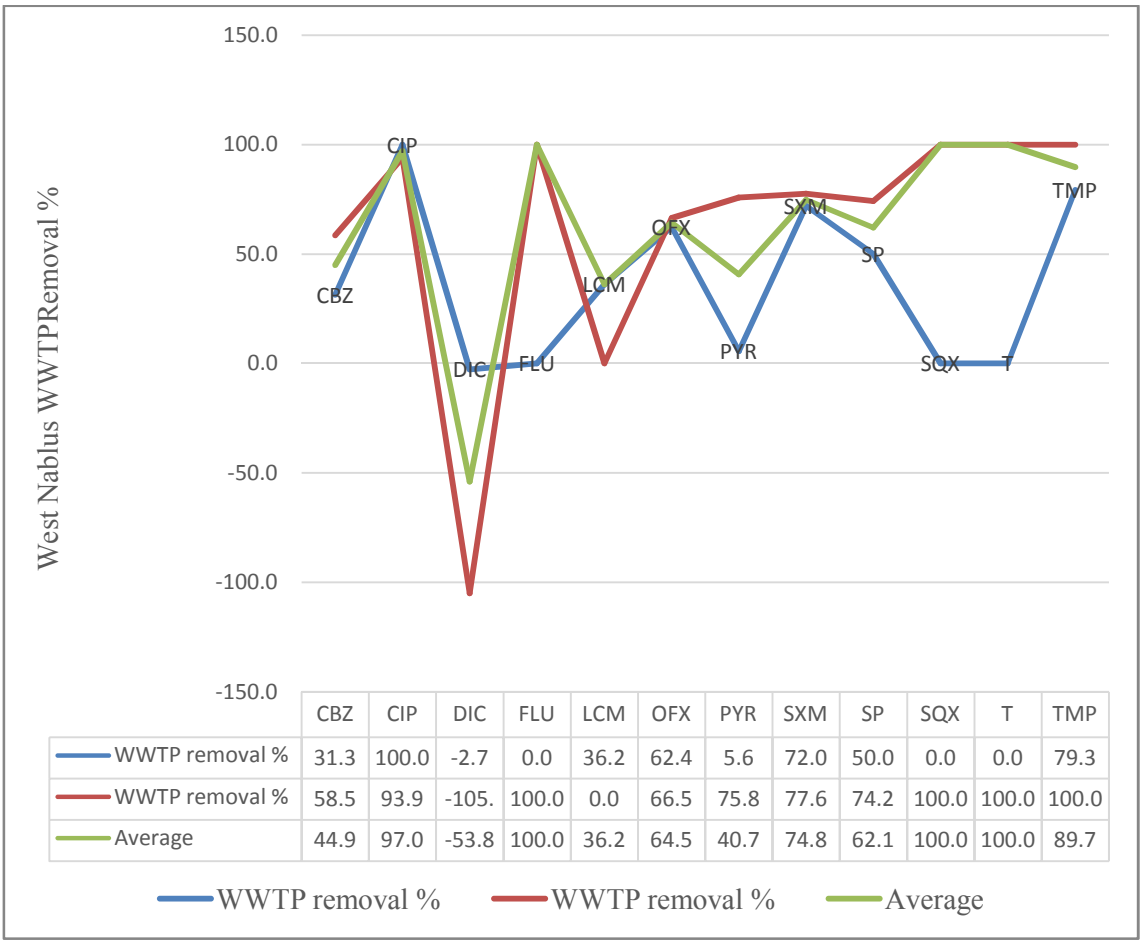


The significant removal efficiency observed in the first campaign is again present in the second campaign. The WWTP showed complete removal of 4 PhCs; SQX, TMP, Flumequine (FLU), and Testosterone, and very high removal for CIP (94%). While it showed fairly good removal capacity for SXM (78 %), PYR (76%), and SP (74%). OFX and CBZ were removed at a rate of 66.5 % and 59%, respectively.

Whereas DIC, interestingly, displayed a higher level of detection than what was present in the inlet sample; the concentration was doubled which resulted in negative removal efficiency of -105%.

Figure 3.3

West Nablus WWTP removal efficiency (%) in both sampling campaigns



	CBZ	CIP	DIC	FLU	LCM	OFX	PYR	SXM	SP	SQX	T	TMP
WWTP removal %	31.3	100.0	-2.7	0.0	36.2	62.4	5.6	72.0	50.0	0.0	0.0	79.3
WWTP removal %	58.5	93.9	-105.	100.0	0.0	66.5	75.8	77.6	74.2	100.0	100.0	100.0
Average	44.9	97.0	-53.8	100.0	36.2	64.5	40.7	74.8	62.1	100.0	100.0	89.7

Figure 3.4 details the variable removal patterns by west Nablus WWTP observed in both campaigns with respect to detected PhCs. A better removal is noticed in the autumn campaign (brown line) than what's noticed in the spring campaign (blue line). Negative removal is observed for DIC in both campaigns, however, it was higher during the autumn. Complete removal was noticed only for CIP in the spring sample and for TMP in the autumn.

9 PhCs are detected in the treated surface wastewater sample collected from wadi Zomar in Anabta in the autumn. The highest concentrations are in descending order: DIC (0.436 µg/L), CBZ (0.4 µg/L), SXM (0.257 µg/L), OFX (0.121 µg/L), and CIP (0.54 µg/L).

FLU and TMP are detected in trace levels, 0.019 and 0.014 µg/L respectively. However, there was no significant spatial difference between the detected

concentrations of PhCs in the WWTP outlet sample and treated surface wastewater in wadi Zomar at the Anabta sampling site of the autumn sample (Wilcoxon signed rank test, p = 0.646).

3.3 POCIS Results

The POCISs revealed the presence of 4 PhCs which are displayed in Figure 10. CBZ had the highest concentration detected followed by DIC, SXM, and CIP in descending order respectively. The POCISs are merely done as screening tests for the presence or absence of any PhCs in groundwater at Anabta.

Figure 3.4

PhCs detected in POCIS sampler (ng/POCIS) deployed at Anabta drinking groundwater reservoir in April/2022 for 28 day

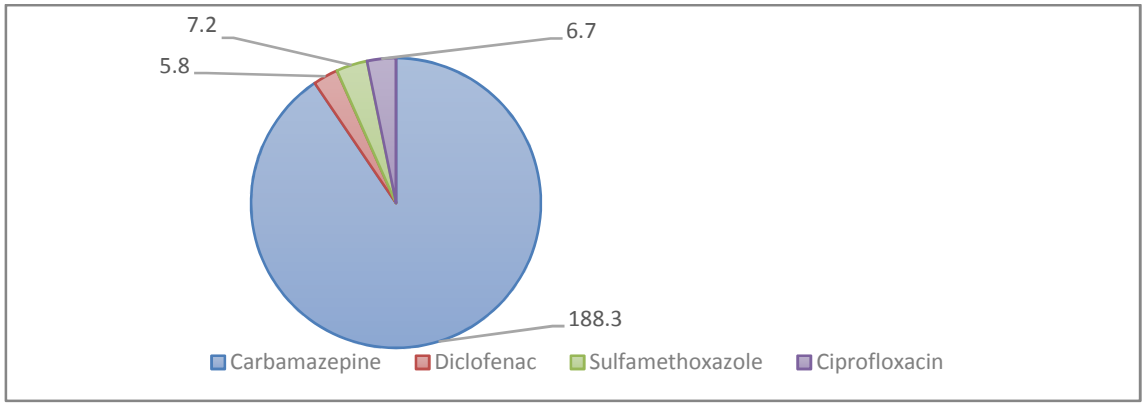


Table 3.2

Summary of Grab Sampling Campaigns results

	Inlet WWTP[1] (µg/L)		Outlet WWTP (µg/L)		Treated surface WW[2] (µg/L)		WWTP average removal efficiency %
PhCs	**First sampling**	**Second sampling**	**First sampling**	**Second sampling**	**First sampling**	**Second sampling**	
Carbamazepine	0.806	1.044	0.554	0.433	0.529	0.4	44.9
Ciprofloxacin	8.043	0.312	0	0.019	0	0.054	97.0
Diclofenac	0.936	0.498	0.961	1.021	0.872	0.436	-53.8
Erythromycin	0	0	0	0	0.022	0	0.00
Flumequine	0	0.029	0	0	0	0.019	100.00
Lincomycin	0.058	0	0.037	0.01	0.015	0	36.2
Ofloxacin	2.67	0.824	1.002	0.276	0.584	0.121	64.5
Pyrimethamine	0.232	0.422	0.219	0.102	0.045	0.09	40.7
Sulfaguanidine	0	0	0.007	0	0	0	0.00
Sulfamethoxazole	0.95	0.818	0.266	0.183	0.143	0.257	74.8
Sulfapyridine	0.266	0.365	0.133	0.094	0.037	0.078	62.1
Sulfaquinoxaline	0	0.031	0.013	0	0.011	0	100
Testosterone	0	0.09	0	0	0	0	100.00
Trimethoprim	0.092	0.055	0.019	0	0.021	0.014	89.7

[1] WWTP: Wastewater Treatment Plant, [2] WW: wastewater, [3] ND: Not-detected (below the detection limit of 0.005 µg/L)

Table 3.3

Physiochemical properties of detected pharmaceuticals

Pharmaceutical	Molecular Weight g/mol	pKa[1]	Log Kow[2]	Water solubility (mg/L)	CAS[3] Registry Number	Chemical Formula
Carbamazepine [4]	236.3	13.9	2.45	18.0	298-46-4	$C_{15}H_{12}N_2O$
Diclofenac[4]	296.1	4.15	4.51	2.37	15307-86-5	$C_{14}H_{11}Cl_2NO_2$
Sulfamethoxazole[4]	253.3	pKa_1: 1.6 pKa_2: 5.7	0.48	610	723-46-6	$C_{10}H_{11}N_3O_3S$
Ciprofloxacin[4]	331.34	acidic 6.09 basic 8.74	0.28	$3 * 10^5$	85721-33-1	$C_{17}H_{18}FN_3O_3$
Erythromycin[4]	733.9	8.9	3.06	F4.2	114-07-8	$C_{37}H_{67}NO_{13}$
Lincomycin[4]	406.5	7.6	0.2	927	154-21-2	$C_{18}H_{34}N_2O_6S$
Ofloxacin[4]	361.4	pKa_1 5.97 pKa_2 9.28	-0.39	$1.08 * 10^4$	82419-36-1	$C_{18}H_{20}FN_3O_4$
Pyrimethamine[4]	248.71	7.34	2.69	10	58-14-0	$C_{12}H_{13}ClN_4$
Sulfaguanidine[4]	214.25	[5]pKa1: 2.21 pKa_2: 11.97	-0.99	$2.6 * 10^5$	57-67-0	$C_7H_{10}N_4O_2S$
Sulfapyridine[4]	249.3	8.43	0.35	268	144-83-2	$C_{11}H_{11}N_3O_2S$
Sulfaquinoxaline[4]	300.34	5.1	1.68	7.5	59-40-5	$C_{14}H_{12}N_4O_2S$
Trimethoprim[4]	290.3	7.12	0.91	400	738-70-5	$C_{14}H_{18}N_4O_3$
Flumequine[4]	261.3	6.5	1.6	2190	42835-25-6	$C_{14}H_{12}FNO_3$
Testosterone[4]	288.4	19.09	3.32	23.4	58-22-0	$C_{19}H_{28}O_2$

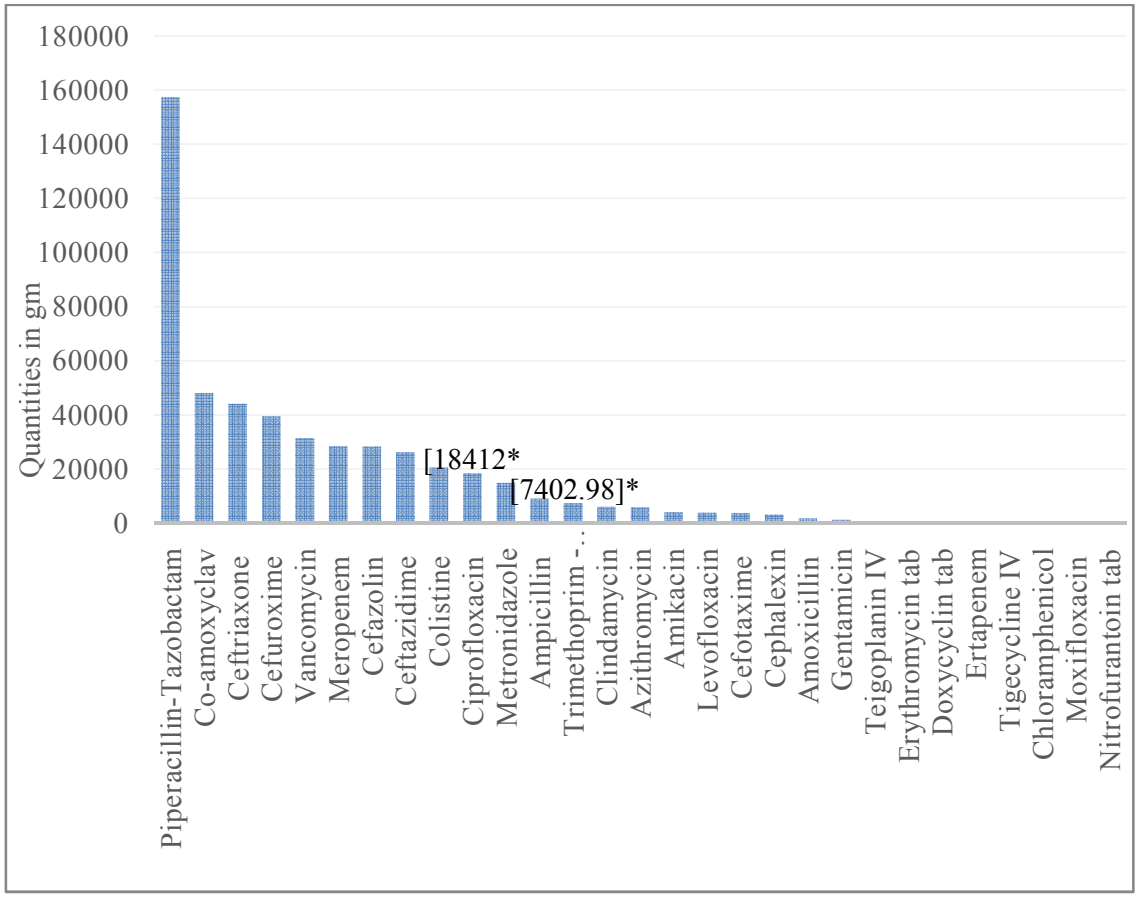
[1]pKa: acid dissociation constant, [2] Log Kow: Log of the octanol/water partition coefficient, [3]CAS Chemical Abstracts Services, [4] Parameters are adopted from PubChem, and ChemSpider,[5] as reported by *Zrnčić et al.* 2015(63)

3.4 Antibiotics dispensary data

The collected dispensary data from all participating hospitals are in figure 3.6 Data were collected for the period 1/1/2021 – 28/2/2022.

Figure 3.5

Cumulative quantities of Antibiotics (in grams) administered for patients from included hospitals of the time period 1/1/2021-28/2/2022.



The above listed antibiotics are the top 15 antibiotics prescribed in the participated hospitals in cumulative order and the next (Amikacin, Levofloxacin, Cephalexin, Amoxicilin, Gentamicin, Teigoplanin, Erythromycin tab, doxycycline tab, Ertapenem, Tigecycline, Chlorampenicol, Moxifloxacin and Nitrofurantoin tab) had a prescribed quantities in the range < 5000 gm in the same studied period.

Cumulative quantities highlighted in red (18412 and 7403 gm) belong to the two classes of antibiotics which were detected in this study Ciprofloxacin and Trimethoprim-Sulfamethoxazole, respectively.

3.5 Promoting AMR development

We estimated the RQ (MEC/lowest PNEC value) according to the antibiotics class where threshold values were developed (Table 3.4). Not all detected antibiotics here in our study have a corresponding threshold value by which an RQ can be calculated.

Ofloxacin residual concentrations in WWTP influent, effluent, and surface wastewater of both campaigns are found to pose risk for AMR development concerning the bacterial community of the WWTP and the environment (RQ>1). The residual concentrations of OFX exceed its respective PNEC of 0.5 µg/L.

Ciprofloxacin concentration in the WWTP influent poses risk for resistance development for the bacterial community employed in the WWTP in both campaigns (RQ>1). As the detected residues in both influent samples are greater than the PNEC of 0.06 µg/L.

For the remaining antibiotics with no corresponding PNEC value, a conservative threshold of 0.05 µg/L is used as guided by the AMR Industry Alliance. Sulfapyridine and Pyrimethamine are found to have a RQ > 1. The measured concentrations of the following samples: WWTP influent and effluent samples of both campaigns and the treated surface WW sample of the autumn campaign are found in concentrations greater than 0.05 µg/L.

Table 3.4

Comparison between PNEC-MIC values provided by Bengtsson-Palme, Larsson et al. (52) and PNEC-ENV by Le page et al. (63) as endorsed by the AMR Industry Alliance and average concentrations of detected antibiotics in the WWTP effluents of the present study

Antibiotic	PNEC-MIC (µg/L)	PNEC-ENV (µg/L)	Lowest value	Inlet concentration Spring (µg/L) Autumn (µg/L)	Outlet concentration Spring (µg/L) Autumn (µg/L)
Ciprofloxacin	0.06	0.45	0.06	8.043 0.312	0 0.019
Flumequine	NA	0.25	0.25	0 0.029	0 0
Lincomycin	2.0	0.81	0.81	0.058 0	0.037 0.01
Ofloxacin	0.5	10.0	0.50	2.67 0.824	1.002 0.276
Sulfamethoxazole	16.0	6.6	6.60	0.95 0.818	0.266 0.183
Trimethoprim	0.50	928.00	0.50	0.092 0.055	0.019 0

PNEC-MIC, ENV: Predicted no environmental concentration-minimum inhibitory concentration, environmental, respectively.µg/L: microgram per liter.

Chapter Four

Discussions and Conclusions

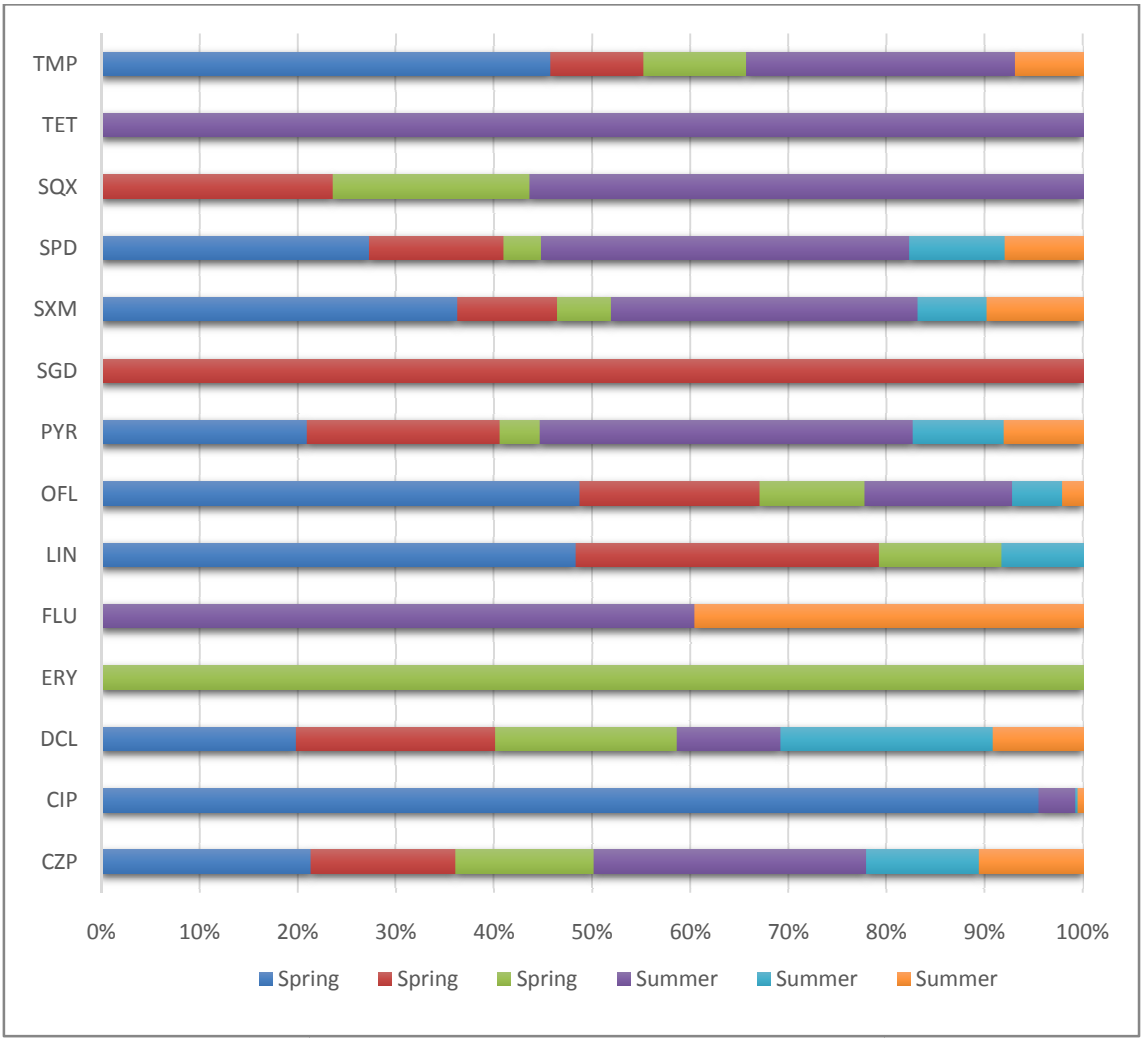
4.1 Occurrence of PhCs in wastewater samples and West Nablus WWTP removal efficiency

The investigation into the occurrence of antibiotics and other pharmaceuticals in sample locations from the study area showed the presence of several PhC residues with varied concentrations. Results also reflected a significant spatial difference between the three sampling locations in the spring season. The autumn campaign results showed only significant removal efficiency by the WWTP of detected PhCs residues and no significant spatial difference between the concentrations of detected PhCs residues detected in west Nablus WWTP effluent and Anabta-Zomar point of sampling.

The results showed that the highest detection level for single PhC in wastewater samples was Ciprofloxacin (8.043 µg/L) which was detected in the influent of the first sampling campaign (spring), while Diclofenac of (1.021 µg/L) was detected in the effluent during the autumn season. In addition, Ofloxacin of (1.002 µg/L) was detected highly during the spring season in the effluent. Moreover, Diclofenac was found to be the highest residue of (0.872 µg/L) in surface-treated wastewater of the spring season sample. We also noticed that OFX residual concentrations detected may aid in the development of OFX resistance.

Figure 4.1

Residual concentrations of different pharmaceutical in the influent, the effluent of the west Nablus WWTP and Anabta-Zomar in both sampling campaigns



The variation of detected PhCs and antibiotics (Figure 4.1), both qualitatively and quantitatively, can be accredited to: the variation in administration and disposal of the detected PhCs concerning the study area, the different physiochemical properties of detected PhCs play a role in their persistence, west Nablus WWTP removal efficiency, abiotic- and biodegradation contributions, different tributaries along Wadi Zomar, agricultural and veterinary activities in the catchment area and the sporadic raw sewerage effluents along Wadi Zomar. The fate of each pharmaceutical compound is determined by a single or a combination of the aforementioned factors.

All hospitals in Nablus send their untreated medical wastewater to the West Nablus WWTP, where it is assumed that the concentration of antibiotic residues will be higher than in any other locality. It somewhat reflects the local protocols used in various

institutions as well as the trend. However, it is interesting to note that some classes of the found antibiotics here are not among the antibiotics that are typically recommended for human administration, according to data gathered from hospitals in the study area. This instead implies that additional sources, such as veterinary and industrial operations, are involved in this. Sadly, local laws governing the use of antibiotics in veterinary practice are not well-applied. (64).

The majority of detected PhCs here share one property that favors their detection in raw wastewater; up to a certain degree, a given dose is excreted, species-dependent, unchanged in the urinary system or through the biliary system based on the route of administration(5) (Table 4.1). Adding to that, most PhCs noticed here are commonly used between both the human and veterinary field.

Furthermore, the variation of PhCs residuals here can be attributed to seasonal variations of infections or medical conditions which require the administration of those specific PhCs. This is noticeable by the markedly high CIP residue in the WWTP influent of the spring sample in comparison to the autumn sample (26 times higher). The same is observed for DIC and OFX (2 and 3 times) higher than the autumn residues, respectively. *Pitarch et al.* reported the seasonal variation in the detection of different classes of PhCs in wastewater favoring the winter season(17). As this mainly could be attributed to more respiratory infections in the winter season requiring medication. In addition to less water consumption; together deliver a precipitation effect.

Table 4.1

General overview regarding the most frequently detected pharmaceutical compounds in our study modified after

PhCs	Acronym	Class	Indication	Excretion
Carbamazepine	CBZ	Anticonvulsant	Epilepsy, Trigeminal Neuralgia, and Psychosis	Metabolites in urine, small percentage as unchanged parent drug
Diclofenac	DIC	Analgesic	Pain relief and fever reducer	*In humans*: 60-70% excreted in the urine. 30% in feces as metabolites and very little as the unchanged parent compound. *In animals:* species dependent.
Ciprofloxacin	CIP	Antibiotics Fluoroquinolones	Broad spectrum antibiotic. Respiratory tract infections Urinary tract infections.	Orally: 40-50% in an unchanged active drug, 15% as metabolites in urine. Parenterally: up to 70% unchanged and 10% as metabolites within 24 hours.
Ofloxacin	OFX	Antibiotics Fluoroquinolones	Broad spectrum antibiotic. urinary tract infections and soft tissue infections colibacillosis in poultry	Orally: 65-80% unchanged in the urine within 48 hours while biliary excretion accounts for 4-8% only.
Flumequine	FLU	Antibiotics Fluoroquinolones	*In humans:* uncomplicated urinary tract infection. *Animals:* enteric infections in food animals and treatment of bacterial infections in farmed fish	*Species dependent:* in veal calves 3.2–6.5% excreted in the urine unchanged. In eels: long elimination time periods.
Lincomycin	LCM	Antibiotics Lincosamide	Gram-positive cocci and anaerobes, except Enterococcus spp. In animals: Bacterial pyoderma Arthritis and pedal osteomyelitis	*In Humans*: no longer used. *In animal*: Variable elimination rate in dairy cattle, bovine, buffalo calves, pigs, cats, and chickens.
Trimethoprim-Sulfamethoxazole	TMP - SXM	Antibiotics Sulfonamides	Broad spectrum antibiotic used in combination with trimethoprim in synergy. Genitourinary, respiratory, and gastrointestinal tract infections. Prophylaxis (in HIV patients) and treatment of Pneumocystis carinii pneumonia.	SXM: Up to 30% excreted in urine as unchanged parent drug. TMP: almost completely excreted unchanged in the urine.
Sulfapyridine	SP	Antibiotics Sulfonamides	As a moiety of Sulfasalazine for Rheumatoid Arthritis Anti-coccidiosis affecting chickens	Mainly unchanged in feces
Sulfaquinoxaline	SQX	Antibiotics Sulfonamides	Anti-coccidiosis agent in poultry. Anticoagulant-based rodenticide.	Mainly excreted in urine
Pyrimethamine	PYR	Antiparasitic	*In human*: chloroquine-resistant malaria and toxoplasma gondii. *In animals*: anti-coccidiosis.	Mainly excreted through the biliary system in the feces.

Note: (83,105,114–120,106–113)

In 2022, the world started to recover from the COVID-19 pandemic where the prescription of antibiotics in COVID-19 patients was high even though evidence for co-bacterial infection was low. Where FQs ranked first among all studied regions as the antibiotic class of choice prescribed for COVID-19 patients(65). The World Health Organization (WHO) categorized FQs among the watch list upholding the antibiotics with the highest potential for resistance development. The watch list should be used in specific infectious diagnoses only and not as an empirical therapy (66). The residual concentrations detected for CIP and OFX in this study and their association with AMR development are discussed later.

In contrast, PYR, CBZ, and SP had a higher detection level in the autumn sample (0.422, 1.044, and 0.365 µg/L), respectively, relative to the spring sample (0.232, 0.806, and 0.266 µg/L), respectively. This could be related to the unregulated and injudicious use of antibiotics and PhCs detected here where a great deal of PhCs are considered OTC. However, according to the authors' knowledge, the available scientific evidence indicating any seasonal variations in local infections which may indicate the administration of the most frequently detected PhCs here is absent.

LCM detection was common among wastewater samples collected in the spring season with concentrations of (0.058, 0.037, and 0.015 µg/L) in WWTP influent, WWTP effluent, and surface wastewater in Wadi Zomar at Anabta sampling point, respectively. While LCM was detected in the WWTP effluent sample of 0.010 µg/L of the autumn campaign. LCM common detection in all samples of one campaign (spring) relative to the other campaign (autumn), can be attributed to the seasonality of dairy products manufactured by cattle and sheep in the spring (67), where Lincomycin is used to treat mastitis (68). LCM is excreted mainly unmetabolized in the urine, feces, and bile and detected in manure, which can be easily dispersed into the environment(69).

The detection of LCM is mainly attributed to its free solubility in aquatic mediums of alkaline pH and thus its resistance to removal. In addition, this could be attributed to the negative competition effect of inorganic cationic molecules (present in Wadi Zomar) on the sorption ability of Lincomycin onto cationic sites in the soil. Furthermore, the poor

sorption ability is enhanced by the increase in pH values beyond the disassociation constant range (70).

Another finding concerning the autumn campaign is the residual concentrations detected for Flumequine. It was detected in the WWTP inlet sample and surface wastewater with a residual concentration of 0.029 µg/L and 0.019 µg/L, respectively. Despite the fact it wasn't detected in the WWTP outlet autumn sample, surface wastewater showed its presence reflecting an introductory source other than the treated wastewater discharged from the WWTP. Wadi Zomar has many scattered tributaries that deliver untreated wastewater that transports along the Wadi. Whereas the local administration of Flumequine is unknown, this FQ is mainly used for enteric infections in animals and in fish farming and also reported to have mutagenic side effects (71).

The WWTP removal efficiency is variable for each compound here (Figure). CIP removal in both sampling campaigns (> 90%), while OFX, SP, SXM, and TMP maintained a removal efficiency in both seasons of a rate of >50%. As opposed to DIC which showed increased concentrations in effluent samples of both campaigns. The main mechanisms by which PhCs are removed can be attributed to adsorption, biodegradation, and /or photolysis.

Ciprofloxacin removal was 100% in the spring and 94% in the autumn. This removal efficiency by the WWTP can be the result of adsorption to sludge employed in the WWTP or the biodegradation by microorganisms used in the WWTP operations. *Bisognin et al.* reported the isolation of OFX and CIP from both the effluent and the sludge of a large WWTP in southern Brazil, with more than 83% of CIP and OFX removed (72).

Carbamazepine was detected in all wastewater samples investigated here with the inability of the WWTP to remove it completely. Furthermore, the removal rate was poor in the first campaign (31%) and increased in the autumn campaign to (59%). Moderate removal can be reached by adsorption into particles of sludge and soil (73). This explains the relatively stable concentration of CBZ in both the WWTP effluent and Wadi Zomar surface wastewater samples of both sampling campaigns. West Nablus

WWTP removal efficiency for CBZ here may be considered a better removal yield in comparison to what *Al-Mashaqbeh et al.* reported for CBZ (22.5%) in Jordan (2018) (12), and even better removal yield than what was previously reported by *Odeh et al.* (2015) (< 20%) in the same study area (74).

CBZ is a hydrophobic, non-polar compound with very poor solubility in aquatic mediums, hence the establishment of its persistence and stability in various ecosystems. CBZ stability in the environment is noticed as previous research in the same study area detected the occurrence of CBZ in concentrations three times higher than those detected in our study (average 3.046 µg/L) (74).

In addition, a recent study in the investigated area concluded the detection of CBZ, SXM, and TMP in wastewater samples in concentrations higher than our study here (Samhan, S. PADUCO Conference; 2022; ANN).

It's concluded that the WWTP is solely responsible for CBZ removal here whereas the removal down Wadi Zomar is rather negligible (Figure 3.3). CBZ is poorly degraded in nature. It mainly depend on temperature. *Tixier et al.* estimated CBZ half-life in the environment by around 63 days in autumn days(75), while another study concluded that CBZ is persistent to abiotic photodegradation and predicted its half-life time of around 100 days in winter (76).

Taking CBZ as an example in terms of water solubility and the impact it enforces on the fate of a chemical compound in the aquatic mediums and its resistance to biodegradation, OFX removal in both campaigns is rather stable. With a removal of 62 % in the spring season and 67% in the autumn season. This can be attributed to the fact that the solubility of OFX in aqueous mediums is pH dependent (2.8 – 5.5) (77), and the operating conditions of the WWTP lie in the alkaline spectrum (pH >7)(56), indicating OFX resistance to solubility in such alkaline mediums and the partial average removal efficiency is due to adsorption to particles either the sludge or suspended solids in the WW or biodegradation by microorganisms in the WWTP (72).

SP was detected in all wastewater samples with concentrations ranging from 0.037 µg/L in the spring surface wastewater to 0.365 µg/L in the autumn WWTP influent sample.

The WWTP delivered variable removal capacity in both campaigns (50% in the spring vs. 74 % in the autumn). The stability of SP in WW samples and resistance to degradation can be attributed to its moderate water solubility (268 mg/L) and resistance to biodegradation by bacteria at the WWTP. Indeed, the removal ability is mainly related to the adsorptive ability of SP onto an organic matter which is influenced by the pH, type of organic content of the medium they are detected at (78). The photolytic properties of sulfonamides additionally enhance the removal due to sun exposure (estimated half-life 2.6 hours)(20) as its removal improves in the autumn sample.

However, for SP and PYR, the removal down Wadi Zomar in the autumn sampling is marginal at 17%, and 11% in comparison to the removal observed in the spring sample at 72% and 79%, respectively. This variation due to dilutional effect delivered by the rainy season in the spring campaign which led to false higher removal percentages in the spring campaign. Or due to the sporadic sewerage discharges containing investigated PhCs along Wadi Zomar past the WWTP effluent point; as the wastewater quality parameters at the Anabta sampling location displayed increased levels in the autumn (Table (4.1).

TMP, SXM, CIP, and FLU are found to have higher concentrations in the surface wastewater autumn sample than what was measured in the WWTP effluent autumn sample. This reflects the introduction of additional residues of PhCs from different tributaries along Wadi Zomar directly after the discharge from West Nablus WWTP.

Where one may argue that a concentrated effect due to the high autumn temperatures is the causative reason for such difference, which is probably unlikely given the fact that SXM is photo-labile. Due to sun exposure, SXM residual concentration in the surface wastewater autumn sample is expected to be less than what it is detected in the WWTP effluent autumn sample, yet it was 37% more in the surface wastewater autumn sample than what it was detected in the WWTP autumn effluent sample (18).

Another photo-labile compound here is DIC, and its removal down Wadi Zomar is seen to be best in the autumn season (57%) whereas the spring season delivered a rather poor removal down Wadi Zomar (9%). This can be attributed to the rapid photodegradation

of DIC in the autumn times as it's estimated to be less than an hour with more sunlight exposure (Wadi Zomar is 100% sun-exposed). While in winter, it's estimated that DIC undergoes photodegradation in around 5 days (76).

It is noted that West Nablus WWTP displayed not effective removal about (36 %) for LCM during the first sampling campaign. The biodegradability of LCM (with an estimated half-life of 30 hours) is thought to be the driving force for its removal as long as the initial concentration didn't exceed the MIC of the present bacteria(80).

Wastewater treatment relies on microorganisms' ability to break down various organic compounds present in the wastewater to reach a specific threshold before effluent wastewater is discharged into an environmental body. The WWTP is required to uphold certain threshold values as standards. As noted in this study, the WWTP has variable removal efficiencies to the measured PhCs.

On the other hand, some PhCs may exert a negative pressure on wastewater purification processes. *Carucci et al.* stated the significant inhibitory effect of Lincomycin present in wastewater on the nitrification treatment employed in the WWTP, which negatively reflects on the WWTP's overall performance(81). The nitrification treatment is thought to be the main mechanism by which TMP is removed (82), a process by which if the present LCM level had any inhibitory effect, the removal of TMP is further rendered. This requires additional studies regarding the matrix effect of wastewater as a stimulant/inhibitory for various PhCs (81).

Sulfamethoxazole and trimethoprim are found to be detected in all wastewater samples (only TMP is absent from the WWTP autumn effluent sample). The residual concentration of SXM is higher than TMP in all measured samples. In the medical field, the administration of this combination is fixed at a ratio of 5 SXM: 1 TMP to combat antimicrobial resistance (83).

Nonetheless, the detected ratio in wastewater samples exceeds the aforementioned ratio. It's detected in 10:1 in the WWTP spring influent sample and 14:1 in its respective autumn sample. Furthermore, their measured residual levels are higher in the spring WWTP influent sample than in the autumn WWTP influent sample. This reflects the

higher administration of SXM/TMP in the spring season in comparison to the autumn season.

In our opinion, we conclude that the scientific evidence regarding the local infectious status requiring the prescription of this antimicrobial is absent. Moreover, the higher ratios detected favoring SXM that can be attributed to the sole administration of SXM alone and not in the form of combination therapy.

The removal efficiency delivered by West Nablus WWTP is high for both SXM and TMP and in both campaigns. This is explained due to their biodegradability by microorganisms employed in the WWTP. As other sources of carbon are depleted in wastewater treatment, pharmaceuticals here are utilized as an energy source instead (82).

Interestingly though, antimicrobial resistance to TMP/SXM is exploited by some bacterial spp as a vital source for growth, as it's the case with TMP/SXM-dependent *Pseudomon*as. This antibiotic-dependent strain is isolated from a cystic fibrosis patient. The patient was put on this regimen as prophylaxis, along which resistance has evolved to become the reason for this strain to survive and cause harm to the patient(84). This could be found in the bacteria-rich working environment of the WWTP with chronic exposure to different antibiotics. This theory can't be verified unless microbiological studies with susceptibility profiles are well conducted.

SXM-TMP combination therapy is commonly administered concurrently, and together with their stability and persistence in wastewater, the detection of this combination in wastewater can be exploited as a pollution marker (85). Sustained surveillance systems would permit the allocation of advanced wastewater treatment processes where most needed according to the major source contributing to their introduction in the wastewater.

A proposed approach for the removal of PhCs from wastewater prior to discharge into the environment is the use of laboratory-engineered, bacterial spp. capable of degrading a selection of antimicrobials as needed(82). As if using AMR backward for better overall health using an environmentally friendlybacterial spp. However, a delicate

balance should be considered when such a solution is implemented as spreading this resistant bacterium has additional side effects we are hardly able to control.

The detected residues in the WWTP effluent have demonstrated a decreased level for all PhCs than what was detected in the WWTP influent in both campaigns except for Diclofenac. The residual level of DIC in the WWTP influent of the spring sample (0.936 µg/L) has increased to (0.961 µg/L) in the WWTP effluent, a marginal increment for the spring effluent sample yet it was further noticed in the autumn campaign.

DIC has increased from (0.498 µg/L) in the WWTP autumn influent to (1.021 µg/L) in the WWTP autumn effluent sample. This can be attributed to several factors of which the increase in effluent concentrations of some PhCs is assigned to the reverse conversion of the drug metabolites into its parent compound through the different enzymatic processes and microbial activity in the plant rather than the introduction of new DIC residues (86)

According to research results here, this effect ranged from almost negligible to doubling the initial concentration detected. Adding to that, the complex matrix present in the wastewater influences the stability and removal of existing chemical compounds (87). The operating conditions of the WWTP highly influence the removal of DIC from wastewater. Which requires acidic pH and prolonged contact time with sludge (87). Whereas the working conditions of the WWTP are in the range of neutral to slightly alkaline. In addition, DIC is resistant to biodegradation by bacteria when other sources of carbon are present(88), as it's the case with high COD levels present in influents of the WWTP.

Another finding regarding the detection of PhCs in the effluent samples which were not detected in the influent samples is LCM of (0.010 µg/L) in the autumn campaign and Sulfaguanidine and Sulfaquinoxaline of (0.007 and 0.013 µg/L), respectively, in the spring effluent sample. Whereas the discrepancy seen in both campaigns could be attributed to either the nature of the momentary effect in grab sampling or it can be attributed to the reversal of the glucuronic conjugate antibiotic metabolites to its parent drug during treatment(89). The PhCs SGD and SQX are veterinary sulfonamides used

as anticoccidials in poultry and cattle. SGD and SQX physiochemical properties indicated by studies on sorption into different types of soil are governed by the increase in organic content and decreased with increased pH and ionic strength (90,91).

Overall, we noticed that the removal efficiency by the WWTP in the autumn campaign was higher than the spring campaign. This could be related to the higher temperature of the autumn season which acted as a more accelerating factor for better removal efficiency as well (79).

As the physiochemical structures of any chemical compound determine its fate in the fluid it's being detected at, the fluid movement plays a role as well in the adsorption and desorption process at sediment materials covering both flanks of Wadi Zomar or suspended particles in the WWTP. With water movement, the adsorbed PhC can be liberated from the adsorbate material and get re-introduced into Wadi Zomar flow rather than adding new residues of the PhCs. The findings of our study are consistence with what is reported elsewhere in the literature (92).

4.2 Occurrence of PhCs in groundwater

The PhCs investigated in this study were also screened for in groundwater as we considered it the vertical end point that residues can reach. Four classes are detected that comply with the most frequently detected PhCs in the wastewater samples. Those are CBZ, DIC, CIP, and SXM (188.3, 5.8, 6.7, and 7.2 ng/POCIS, respectively). The groundwater and tap water, on a global scale, are found to harbor 16 different PhCs in several countries as well. Where Carbamazepine, Diclofenac, Sulfamethoxazole, Ibuprofen, and Naproxen are most frequently detected worldwide and in different aquatic and soil compartments (21).

The WWTP effluent in both campaigns had residual concentrations of the aforementioned PhCs (except CIP in the spring effluent), their residues are further noticed in the surface wastewater sample at the Anabta-Zomar sampling location indicating the sorption of PhCs into the soil of Wadi Zomar and along it's both banks. Even though the soil hasn't been screened for PhCs here, the residuals detected in groundwater imply no other alternative. It's found that soil polluted with high loads of

PhCs in a certain area is accompanied by contaminated groundwater in the same area as well (24).

The location of the treated surface wastewater sample in Anabta-Zomar is between two groundwater wells utilized as regular groundwater (Figure). Water recharge is crucial in maintaining the groundwater sources in this area as it's the sole source of viable groundwater. In addition, Wadi Zomar natural path is situated in close proximity to the aquifers of the studied location, combining that with the hydrological structure of the area, the discharged-WWTP effluent, the sporadic discharge of untreated sewage prior and at Anabta village and the agricultural activity of the area; the leach of different contaminants in groundwater sample is observed. For example, in India, Ciprofloxacin was detected (among others) in high alarming levels in water wells (>1 µg/L) in villages near the effluent of a WWTP for 90 bulk drug manufacturers(93). Emphasizing the importance of location in risk assessment.

The significant removal detected in the spring campaign between the WWTP effluent and Anabta-Zomar point of sampling could be attributed to rain events with dilutional effects in addition to sorption and degradation along the Wadi. Rainfall events aided with the natural hydrological structure of Wadi Zomar in the studied area (57,59), and the chronic endurance of such PhCs making groundwater pollution a question of time; Piston-effect.

It's reasonable to deduce the presence of a tight link between the occurrence of PhCs in treated wastewater and surface water. The leach of different classes of PhCs here is assigned to the adsorption potential of each compound and is resistant to biodegradation (94). SXM, CBZ, and DIC share low water solubility (Table 3.3).

The ingestion of water contaminated with different classes of antibiotics here raises concerns regarding the potential health effects for humans and animals. Concerns are at a point here regarding the risk associated with the wide usage of groundwater by all study area population age groups. The very young and very old are considered vulnerable sub-populations and more sensitive to any change in the gut microbiome.

This change is associated with daily intake of pharmaceutically polluted groundwater (27).

The detection of different classes of PhCs in treated surface wastewater and groundwater is mainly attributed to wastewater effluents in the receiving environment. The literature review points towards the application of further treatment of wastewater for removal of different pharmaceutical residuals including antibiotics (76,95–99) which requires the application of advance infrastructure for the WWTP (Oxidation &/ Ozonation coupled with UV application), membrane bioreactors or simply solar systems (100). However, this is governed by the status of the local area regarding which PhCs are in great need of removal. As groundwater is contaminated with the most frequently detected PhCs here, urgent mitigation protocols are required.

4.3 Potential Health effects of antibiotics measured environmental concentrations and the development of AMR

The main critical health effect of high residual concentrations of antibiotics in the environment is the development of antimicrobial resistance. Table 3.4 presents the detected residuals of antibiotics in wastewater influent and effluent samples of our study here for comparison against the threshold values of PNEC – MIC developed by *Bengtsson-Palme, Larsson et al.*(45) and PNEC – ENV developed by *Le page et a.l*(46). The lowest threshold value of both PNECs is considered the critical value to determine AMR risk on conservative grounds. We estimated the RQ (MEC/lowest PNEC value) according to the antibiotics class where threshold values were developed. Not all detected antibiotics here in our study have a corresponding threshold value by which an RQ can be calculated (45,46).

According to the concentrations measured in the WWTP influent samples, we noticed a potential for AMR development in the bacterial community involved in the WWTP conventional operations employed for CIP and OFX (RQ > 1). The remaining antibiotics with a corresponding PNEC values were found not to constitute any risk for AMR according to the residuals detected (RQ <1). Nonetheless, the chronic effect due to prolonged exposure times is yet needed.

CIP of 8.043 µg/L and OFX of 2.67 µg/L in the WWTP spring influent sample and CIP of 0.312 µg/L and OFX of 0.824 µg/L in the WWTP autumn influent sample are all found in concentrations exceeding the lowest PNEC threshold value in both seasons. This alerts for studying the association between the detection of highly alarming residual concentrations of antibiotics and the presence and extent of FQ's resistant genes/bacteria in effluents and the sludge. As the discharged effluent is being disinfected before releasing it into Wadi Zomar, evading disinfection is still a possibility (40).

Water provides a rather supporting medium where resistance can get allocated easily. Furthermore, it supports its persistence and widespread detection(28). The wastewater medium is considered a nutrient-rich habitat for both sensitive and resistant bacteria to proliferate. Horizontal gene transfer can easily happen and mobile genetic elements (plasmids and transposons) can be exchanged in the gene pool. For instance, the high abundance of antibiotics residues (e.g. Macrolides with a range of 0.1-10 ng/L, and FQs range of 0.10- 1000 µg/L) in the influent of a WWTP was found to be significantly positively associated with the genetic material responsible for their resistance(30).

Additionally, it's found that only OFX residual concentration in the WWTP spring effluent sample poses risk for AMR development (RQ >1) against clinical bacteria present in the environment but not a risk for the environmental bacteria itself. The detected spring residual concentration of OFX (1.002 µg/L) exceeds the lowest threshold values for the PNEC-MIC and PNEC-ENV which is the PNEC-MIC value (0.5 µg/L). This is in agreement with what *Booth et al.* concluded in their retrospective study which indicated that OFX was among the most frequently detected antibiotic in concentrations exceeding PNEC-MIC in surface water while CIP ranked first in percentages of analyses exceeding the PNEC-MIC on a global scale (101).

This is of importance as Wadi Zomar naturally passes through populated and active agricultural lands on both of its flanks. This is a worrisome point here considering the potential threat of effluents in disseminating resistance to the environment impacting human and animal health and agricultural life forms. A previous study yielded the detection and enhanced survival of clinically relevant bacterial spp. (*E. coli,*

Pseudomonas, Klebsiella and *Enterococcus*) in sediments of Wadi Zomar which precludes the possibility of detection of resistant strains as well(59).

Moreover, resistant gram-negative bacteria were detected in in-house, greywater samples collected from farms in the West Bank, Palestine, which *Craddock et al.* had investigated. In their study, resistance was reported mainly for ampicillin followed by TMP-SXT, tetracycline, and cefazolin in decreasing order. Additionally, an alarming finding is the detection of multi-drug resistance isolates (~8%) as well. The effluent which was found to harbor resistant isolates is used traditionally for crop irrigation to cover the water scarcity (29).

The RQ of OFX is more than 1 in spring surface wastewater when compared to the residual concentration.. The residual concentration of (0.584 µg/L) in the surface wastewater sample at the Zomar-Anabta point of sampling reflects a possibility for resistance development and dissemination. In comparison to the autumn sample, the RQ < 1. The slightly increased removal capacity delivered by the WWTP in the autumn (66%) has aided in decreasing the residual concentration in the WWTP effluent prior to discharge to the concentration of (0.276 µg/L). This level, if not increased, to our belief and based on the literature threshold values, holds no risk for AMR in the bacterial community of Wadi Zomar in the autumn. On the other hand, the removal effect for OFX down Wadi Zomar in the autumn additionally lowered OFX residue by a 56%. Rendering the ability for AMR emergence.

Combining the detection of residual antibiotics here in levels promoting selection pressure in WWTP effluent samples and the physiochemical properties that permits antibiotics to sustain their persistence in the wastewater samples collected with enough exposure time, then the possibility for AMR genes/bacteria is high. Furthermore, in *Hanna et al.* study, they found that the ESBL-coding gene and plasmid-mediated quinolone-resistance gene are the most frequently detected in river water and sediment over a three-year study(102). *Guo et al.* reported the vast abundance of ARGs in dewatered sludge, where ARGs copies were detected in the final effluent with varying concentrations in comparison to the influent. ARGs are influenced by both the bacterial community and the residual concentration of antibiotics present (30).

In this study, the microbiological profile of the wastewater samples collected wasn't investigated yet the detection of antibiotics itself might be indirectly an indicator for the presence of AMR bacteria and AMR genes have-given their common excretion source (3,7,11).

The RQ for SP and PYR is found to be > 1 in the WWTP influent and effluent samples of both campaigns. This was noticed when comparing the MEC of SP and PYR in the influent and effluent samples with a default threshold value of (0.05 µg/L) proposed by the AMR Industry Alliance (47). However, their role in promoting AMR is unknown; health problems arising from the present trace residuals are lagging, and the development of target threshold values is still ongoing. In our opinion, this suggests a more strict and protective approach for adhering to a target threshold of zero µg/L before any effluent is discharged into the environment to avoid any harm.

Even though there is a theoretically proposed limit for resistance development in clinical bacteria, the complex matrix of the aquatic environment has its print (103,104)**.** It has been observed in ecotoxicological studies for drugs, DIC, and CBZ, in which the mixture effect against representative environmental spp is different than when the compounds are tested solely. Over and above that, the single residual for any compound in the mixture was in the range or less than the level which is proposed to have significant toxicity (23).

4.4 Strengths and Limitations

We had two limitations here in our study, one is the sample size and another is the variable occurrence of PhCs which may be attributed to the sampling nature being rather momentary (grab) than composite (i.e. 24 hours sample). On the other hand, this study provides preliminary insights regarding the occurrence of different classes of PhCs and antibiotics that could help in the evaluation of the local status of AMR and eco-toxicity.

4.5 Conclusion and Recommendations

In summary, the findings of this study are in agreement with the published literature regarding the occurrence and level of PhCs in different aquatic compartments.

- Wastewater and groundwater were found to be polluted with PhCs. Urgent mitigation measures are warranted as groundwater is found polluted with different PhCs.
- West Nablus WWTP requires integrating additional technologies to enhance the removal of PhCs.
- Routine surveillance systems are needed to monitor the quality of wastewater with respect to different PhCs. Data produced from surveillance reports can be used to allocate mitigation protocols where most needed and with the most efficient methods.
- Antimicrobial stewardship programs and strategies for PhCs disposal should be followed.
- OFX and CIP residual concentrations are found to be higher than the PNEC-MIC and are in the range of promoting for resistance. The microbiological profile of the bacterial community of the influent and effluent for FQ's resistance genes is needed to further understand AMR status here.

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List of Abbreviations

Abbreviation	Meaning
AMR	Antimicrobial resistance
AGP	Animal Growth Promoters
ARG	Antibiotic-Resistant Gene
BOD_5	Biological Oxygen Demand (5-days)
CAS	Chemical Abstract Services
CIP	Ciprofloxacin
COD	Chemical Oxygen Demand
COVID-19	Corona Virus Disease of 2019
CBZ	Carbamazepine
DIC	Diclofenac
$DDIH_2O$	Distilled Deionized Water
EDS	Endocrine Disturbing Substances
EMA	European Medicines Agency
ERA	Estimated Risk Assessment
ERY	Erythromycin
ESBL	Extended Spectrum Beta Lactamases
EU	European Union
EUCAST	European Committee on Antibiotic Susceptibility Testing
FLU	Flumequine
FQs	Fluoroquinolones
HIV	Human Immunodeficiency Virus
HLB	Hydrophilic Lipophilic Balance
IRB	Institutional Review Board
LC-MS/MS	Liquid Chromatography – Mass Spectrometry/ tandem

Abbreviation	Meaning
	Mass Spectrometry
LCM	Lincomycin
$LogK_{ow}$	Log of Octanol/Water Partition Coefficient
MEC	Measured Environmental Concentration
MIC	Minimal Inhibitory Concentration
MRM	Multiple Reaction Monitoring
MSC	Minimal Selective Concentration
µg/L	Micro-gram per one Liter
OFX	Ofloxacin
OTC	Over The Counter
PEC	Predicted Environmental Concentration
pH	Potential of Hydrogen
PhCs	Pharmaceuticals
pKa	Acid dissociation constant
PNEC	Predicted No Effect Concentration
PNEC - ENV	PNEC – Environmental
POCIS	Polar Organic Chemical Integrated Sampler
PYR	Pyrimethamine
SGD	Sulfaguanidine
SP	Sulfapyridine
SPE	Solid Phase Extraction
spp	Species
SQX	Sulfaquinoxaline
SXM	Sulfamethoxazole
T	Testosterone
TMP	Trimethoprim

Abbreviation	Meaning
TSS	Total Suspended Solids
USA	United States of America
WHO	World Health Organization
WW	Waste Water
WWTP	Waste Water Treatment Plant

References

1. Pereira A, Silva L, Laranjeiro C, Lino C, Pena A. Selected pharmaceuticals in different aquatic compartments: Part II-Toxicity and environmental risk assessment. Molecules. 2020;25(8).

2. Vajda AM, Barber LB, Gray JL, Lopez EM, Woodling JD, Norris DO. Reproductive Disruption in Fish Downstream from an Estrogenic Wastewater Effluent. Environ Sci Technol [Internet]. 2008 May 1;42(9):3407–14. Available from: https://pubs.acs.org/doi/10.1021/es0720661

3. Mohan A, Bashir S, Mohan A, Kumar D, Kaur N. Occurrence and Fate of Antibiotics in Manure. In: Jawaid M, Khan A, editors. Manure Technology and Sustainable Development [Internet]. Singapore: Springer Nature Singapore; 2023. p. 321–39. Available from: https://doi.org/10.1007/978-981-19-4120-7_14

4. Oaks JL, Gilbert M, Virani MZ, Watson RT, Meteyer CU, Rideout BA, et al. Diclofenac residues as the cause of vulture population decline in Pakistan. Nature. 2004 Feb;427(6975):630–3.

5. Wang B, Hu L, Siahaan TJ. Drug delivery: principles and applications. John Wiley & Sons; 2016.

6. Van Boeckel TP, Pires J, Silvester R, Zhao C, Song J, Criscuolo NG, et al. Global trends in antimicrobial resistance in animals in low- And middle-income countries. Science (80-). 2019;365(6459).

7. Haller MY, Müller SR, McArdell CS, Alder AC, Suter MJF. Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. J Chromatogr A. 2002;952(1–2):111–20.

8. Fonseca BB, Fernandez H, Rossi DA. Campylobacter spp. and related organisms in poultry. Campylobacter Spp Relat Org Poult Pathog Interact Diagnosis Epidemiol. 2016;1–206.

9. Adwan G, Isayed H. Prevalence and Characterization of Staphylococcus aureus Isolated from Bulk Tank Milk Dairy Cow Farms in West Bank-Palestine. Microbiol Res J Int. 2018;23(5):1–13.

10. Al-Dawodi R, Farraj MA, Essawi T. Antimicrobial resistance in non-typhi Salmonella enterica isolated from humans and poultry in Palestine. J Infect Dev Ctries. 2012;6(2):132–6.

11. Larsson DGJ. Pollution from drug manufacturing: Review and perspectives. Philos Trans R Soc B Biol Sci. 2014;369(1656).

12. Al-Mashaqbeh, Alsafadi, Dalahmeh, Bartelt-Hunt, Snow. Removal of Selected Pharmaceuticals and Personal Care Products in Wastewater Treatment Plant in Jordan. Water [Internet]. 2019 Sep 26;11(10):2004. Available from: https://www.mdpi.com/2073-4441/11/10/2004

13. You Y, Silbergeld EK. Learning from agriculture: Understanding low-dose antimicrobials as drivers of resistome expansion. Front Microbiol. 2014;5(JUN):1–10.

14. Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, et al. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. Sci Total Environ [Internet]. 2013;447:345–60. Available from: http://dx.doi.org/10.1016/j.scitotenv.2013.01.032

15. Feng L, Cheng Y, Zhang Y, Li Z, Yu Y, Feng L, et al. Distribution and human health risk assessment of antibiotic residues in large-scale drinking water sources in Chongqing area of the Yangtze River. Environ Res [Internet]. 2020 Jun 1 [cited 2021 Sep 14];185:109386. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0013935120302796

16. Serwecinska L. Antimicrobials and Antibiotic-Resistant Bacteria: Water. 2020;12:1–17.

17. Bijlsma L, Pitarch E, Fonseca E, Ibáñez M, Botero AM, Claros J, et al. Investigation of pharmaceuticals in a conventional wastewater treatment plant: Removal efficiency, seasonal variation and impact of a nearby hospital. J Environ Chem Eng [Internet]. 2021 Aug;9(4):105548. Available from: https://linkinghub.elsevier.com/retrieve/pii/S221334372100525X

18. Mahmood AR, Al-Haideri HH, Hassan FM. Detection of Antibiotics in Drinking Water Treatment Plants in Baghdad City, Iraq. Adv Public Heal. 2019;2019(March):1–10.

19. Zessel K, Mohring S, Hamscher G, Kietzmann M, Stahl J. Biocompatibility and antibacterial activity of photolytic products of sulfonamides. Chemosphere [Internet]. 2014;100:167–74. Available from: https://www.sciencedirect.com/science/article/pii/S0045653513016287

20. Challis JK, Carlson JC, Friesen KJ, Hanson ML, Wong CS. Aquatic photochemistry of the sulfonamide antibiotic sulfapyridine. J Photochem Photobiol A Chem [Internet]. 2013;262:14–21. Available from: http://dx.doi.org/10.1016/j.jphotochem.2013.04.009

21. Tim aus der Beek, Frank-Andreas Weber AB, Gregor Grüttner AC. Pharmaceuticals in the environment: Global occurrence and potential cooperative action under the Strategic Approach to International Chemicals Management (SAICM) [Internet]. Vol. 3, German Environment Agency. 2016. 80–91 p. Available from: https://www.umweltbundesamt.de/sites/default/files/medien/1968/publikationen/iww_abschlussbericht_saicm_arzneimittel_final.pdf

22. Dalahmeh S, Björnberg E, Elenström AK, Niwagaba CB, Komakech AJ. Pharmaceutical pollution of water resources in Nakivubo wetlands and Lake Victoria, Kampala, Uganda. Sci Total Environ [Internet]. 2020;710:136347. Available from: https://doi.org/10.1016/j.scitotenv.2019.136347

23. ZIND H, MONDAMERT L, REMAURY QB, CLEON A, LEITNER NKV, LABANOWSKI J. Occurrence of carbamazepine, diclofenac, and their related metabolites and transformation products in a French aquatic environment and preliminary risk assessment. Water Res [Internet]. 2021;196:117052. Available from: https://doi.org/10.1016/j.watres.2021.117052

24. Hussain S, Naeem M, Chaudhry MN. Estimation of Residual Antibiotics in Soil and Underground Water of Areas Affected by Pharmaceutical Wastewater in Lahore 1. 2017;39(1):56–60.

25. Ben Y, Hu M, Zhang X, Wu S, Wong MH, Wang M, et al. Efficient detection and assessment of human exposure to trace antibiotic residues in drinking water. Water Res [Internet]. 2020;175:115699. Available from: https://doi.org/10.1016/j.watres.2020.115699

26. Cleuvers M. Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. Ecotoxicol Environ Saf. 2004;59(3):309–15.

27. Ben Y, Hu M, Zhong F, Du E, Li Y, Zhang H, et al. Human daily dietary intakes of antibiotic residues: Dominant sources and health risks. 2022;212(May).

28. Taylor NGH, Verner-Jeffreys DW, Baker-Austin C. Aquatic systems: Maintaining, mixing and mobilising antimicrobial resistance? Trends Ecol Evol. 2011;26(6):278–84.

29. Craddock HA, Chattopadhyay S, Rjoub Y, Rosen D, Greif J, Lipchin C, et al. Antibiotic-resistant Escherichia coli and Klebsiella spp. in greywater reuse systems and pond water used for agricultural irrigation in the West Bank, Palestinian Territories. Environ Res [Internet]. 2020 Sep;188:109777. Available from: https://www.sciencedirect.com/science/article/pii/S0013935120306708

30. Guo X, Yan Z, Zhang Y, Xu W, Kong D, Shan Z, et al. Behavior of antibiotic resistance genes under extremely high-level antibiotic selection pressures in pharmaceutical wastewater treatment plants. Sci Total Environ [Internet]. 2018;612:119–28. Available from: http://dx.doi.org/10.1016/j.scitotenv.2017.08.229

31. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629–55.

32. Neill JO'. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations The Review on Antimicrobial Resistance Chaired. 2014;(December).

33. Dcosta VM, King CE, Kalan L, Morar M, Sung WWL, Schwarz C, et al. Antibiotic resistance is ancient. Nature [Internet]. 2011;477(7365):457–61. Available from: http://dx.doi.org/10.1038/nature10388

34. Nobrega DB, Tang KL, Caffrey NP, De Buck J, Cork SC, Ronksley PE, et al. Prevalence of antimicrobial resistance genes and its association with restricted antimicrobial use in food-producing animals: A systematic review and meta-analysis. J Antimicrob Chemother. 2021;76(3):561–75.

35. Shoemaker NB, Vlamakis H, Hayes K, Salyers AA. Evidence for extensive resistance gene transfer among Bacteroides spp. and among Bacteroides and other genera in the human colon. Appl Environ Microbiol. 2001;67(2):561–8.

36. Martinez JL. The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proc R Soc B Biol Sci. 2009;276(1667):2521–30.

37. Davies J. Origins and evolution of antibiotic resistance. Microbiologia. 1996;12(1):9–16.

38. Zinger L, Gobet A, Pommier T. Two decades of describing the unseen majority of aquatic microbial diversity. Mol Ecol. 2012;21(8):1878–96.

39. Wells MJM. Log DOW: Key to understanding and regulating wastewater-derived contaminants. Environ Chem. 2006;3(6):439–49.

40. Khan S, Beattie TK, Knapp CW. The use of minimum selectable concentrations (MSCs) for determining the selection of antimicrobial resistant bacteria. Ecotoxicology [Internet]. 2017;26(2):283–92. Available from: http://dx.doi.org/10.1007/s10646-017-1762-y

41. Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, et al. Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. Lipsitch M, editor. PLoS Pathog [Internet]. 2011 Jul 21;7(7):e1002158. Available from: https://dx.plos.org/10.1371/journal.ppat.1002158

42. Murray AK, Stanton IC, Wright J, Zhang L, Snape J, Gaze WH. The 'selection end points in communities of bacteria' (Select) method: A novel experimental assay to facilitate risk assessment of selection for antimicrobial resistance in the environment. Environ Health Perspect. 2020;128(10):107007-1-107007–10.

43. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. Clin Microbiol Rev. 2018;31(4).

44. Agency EM. GUIDELINE ON THE ENVIRONMENTAL RISK ASSESSMENT OF MEDICINAL. 2006;(June):1–12.

45. Bengtsson-Palme J, Larsson DGJ. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. Environ Int [Internet]. 2016;86:140–9. Available from: http://dx.doi.org/10.1016/j.envint.2015.10.015

46. Le G, Gunnarsson L, Snape J, Tyler CR. Integrating human and environmental health in antibiotic risk assessment: A critical analysis of protection goals, species sensitivity and antimicrobial resistance. Environ Int [Internet]. 2017;109(September):155–69. Available from: http://dx.doi.org/10.1016/j.envint.2017.09.013

47. Alliance AI. AMR Industry Alliance target PNEC list [Internet]. 2022. Available from: https://www.amrindustryalliance.org/wp-content/uploads/2022/04/AMR-Table-1-Update-February-2022.pdf

48. Commission Implementing Decision (EU) 2022/1307 of 22 July 2022 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council (notified under [Internet]. 2022. p. 117–20. Available from: http://data.europa.eu/eli/dec_impl/2022/1307/oj

49. Hanna N, Sun P, Sun Q, Li X, Yang X, Ji X, et al. Presence of antibiotic residues in various environmental compartments of Shandong province in eastern China : Its potential for resistance development and ecological and human risk. Environ Int [Internet]. 2018;114(July 2017):131–42. Available from: https://doi.org/10.1016/j.envint.2018.02.003

50. Craddock HA, Panthi S, Rjoub Y, Lipchin C, Sapkota A, Sapkota AR. Antibiotic and herbicide concentrations in household greywater reuse systems and pond water used for food crop irrigation: West Bank, Palestinian Territories. Sci Total

Environ [Internet]. 2020;699:134205. Available from: https://doi.org/10.1016/j.scitotenv.2019.134205

51. Salem HS, Yihdego Y, Muhammed HH. The status of freshwater and reused treated wastewater for agricultural irrigation in the Occupied Palestinian Territories. J Water Heal |. 2021;19:120–58.

52. Silori R, Shrivastava V, Singh A, Sharma P, Aouad M, Mahlknecht J, et al. Global groundwater vulnerability for Pharmaceutical and Personal care products (PPCPs): The scenario of second decade of 21st century. J Environ Manage [Internet]. 2022;320:115703. Available from: https://www.sciencedirect.com/science/article/pii/S0301479722012762

53. Abu Taha A, Atia Z, Naji R. Prevalence and antibiotic susceptibility of bacterial pathogens at a tertiary care hospital in Nablus, occupied Palestinian territory: a cross-sectional survey. Lancet [Internet]. 2019;393:S50. Available from: https://www.sciencedirect.com/science/article/pii/S0140673619306361

54. Qadi M, Alhato S, Khayyat R, Elmanama AA. Colistin Resistance among *Enterobacteriaceae* Isolated from Clinical Samples in Gaza Strip. Detolla L, editor. Can J Infect Dis Med Microbiol [Internet]. 2021;2021:6634684. Available from: https://doi.org/10.1155/2021/6634684

55. Elmanama AA, Albayoumi MA. High Prevalence of Antibiotic Residues among Broiler Chickens in Gaza Strip. Food Public Heal. 2016;6(4):93–8.

56. Abu Jaffal Y, Homeidan M, Bitar S, Abu Salameh R. Wastewater Treatment Plant Nablus West Operation Annual Report. 2023;1–25. Available from: file:///C:/Users/Ala Jaddou/Dropbox/My PC (A-Jaddou)/Downloads/تقرير-شهر-1-2023.pdf

57. Palestinian Water Authority. 2011 .نبذة عن مصادر المياه في فلسطين.

58. Palestinian Water Authority. Water resoureces in state of Palestine [Internet]. [cited 2022 Sep 17]. Available from: http://www.pwa.ps/userfiles/server/خـــرائط سلطة المياه/water-Supply.jpg

59. Khayat S, Sulaiman S, Mimi Z. Using Biological Indicators to Characterize the Natural Flow Regime in Wadi Zomar Stream/Palestine. Asian J Appl Sci 2011. 2011;

60. Bartelt-Hunt SL, Snow DD, Damon T, Shockley J, Hoagland K. The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska. Environ Pollut [Internet]. 2009;157(3):786–91. Available from: http://dx.doi.org/10.1016/j.envpol.2008.11.025

61. Lee SS, Paspalof AM, Snow DD, Richmond EK, Rosi-Marshall EJ, Kelly JJ. Occurrence and Potential Biological Effects of Amphetamine on Stream Communities. Environ Sci Technol [Internet]. 2016 Sep 6;50(17):9727–35. Available from: https://doi.org/10.1021/acs.est.6b03717

62. The Standards and Metrology Organization and the Palestinian Water Authority. Mandatory Technical Instructions 2012-34 Treated water for agricultural irrigation. 2012;1–5. Available from: https://moa.pna.ps/laws

63. Zrnčic M, Babic S, Mutavdžic Pavlovic D. Determination of thermodynamic pKa values of pharmaceuticals from five different groups using capillary electrophoresis. J Sep Sci. 2015;38(7):1232–9.

64. Abukhattab S, Kull M, Abu-Rmeileh NME, Cissé G, Crump L, Hattendorf J, et al. Towards a One Health Food Safety Strategy for Palestine: A Mixed-Method Study. Antibiotics. 2022;11(10):1–21.

65. Langford BJ, So M, Raybardhan S, Leung V, Soucy JR, Westwood D, et al. Antibiotic prescribing in patients with COVID-19□: rapid review and. Clin Microbiol Infect [Internet]. 2021;27(4):520–31. Available from: https://doi.org/10.1016/j.cmi.2020.12.018

66. WHO. WHO releases the 2019 AWaRe Classification Antibiotics [Internet]. 2019. Available from: https://www.who.int/news/item/01-10-2019-who-releases-the-2019-aware-classification-antibiotics#:~:text=The Watch group includes 110 antibiotics%2C 11 of,options for specified infectious syndromes. RESERVE GROUP ANTIBIOTICS

67. Amro A, Mansoor B, Hamarsheh O, Hjaija D. Recent trends in human brucellosis in the West Bank, Palestine. Int J Infect Dis [Internet]. 2021;106:308–13. Available from: https://doi.org/10.1016/j.ijid.2021.04.037

68. McDougall S. Intramammary treatment of clinical mastitis of dairy cows with a combination of lincomycin and neomycin, or penicillin and dihydrostreptomycin. N Z Vet J [Internet]. 2003 Jun;51(3):111–6. Available from: http://www.hc-sc.gc.ca/dhp-mps/vet/mrl-lmr/mrl-lmr_versus_new-nouveau-eng.php

69. Mercer MA. lincosamide in animals [Internet]. MSD Veterinary Manual. [cited 2022 Oct 26]. Available from: https://www.msdvetmanual.com/pharmacology/antibacterial-agents/lincosamides-use-in-animals#

70. Wang C, Boyd SA. Sorption of Lincomycin at Low Concentrations from Water by Soils. Soil Chem. 2011;

71. Kuroda K, Kijima A, Ishii Y, Takasu S, Jin M, Matsushita K, et al. Flumequine enhances the in vivo mutagenicity of MeIQx in the mouse liver. Arch Toxicol. 2013;87(8):1609–19.

72. Bisognin RP, Wolff DB, Carissimi E, Prestes OD, Zanella R. Occurrence and fate of pharmaceuticals in effluent and sludge from a wastewater treatment plant in Brazil. Environ Technol [Internet]. 2021 Jul 3;42(15):2292–303. Available from: https://www.tandfonline.com/doi/full/10.1080/09593330.2019.1701561

73. Chen X, Hu Z, Zhang Y, Zhuang L, Zhang J, Li J, et al. Removal processes of carbamazepine in constructed wetlands treating secondary effluent: A review. Water (Switzerland). 2018;10(10):1–16.

74. Odeh W. Occurrence and Fate of EDCs in Wastewater and Their Receiving Aquatic Environments in the West Bank of Palestine [Internet]. Ben-Gurion University of the Negev, The Jacob Blaustein Institutes for Desert Research, The Albert Katz International School for Desert Studies; 2015. Available from: https://books.google.ps/books?id=p9NqAQAACAAJ

75. Tixier C, Singer HP, Oellers S, Müller SR. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. Environ Sci Technol. 2003;37(6):1061–8.

76. Andreozzi R, Marotta R, Paxéus N. Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. Chemosphere. 2003;50(10):1319–30.

77. Al-Omar MA. Chapter 6 Ofloxacin. Vol. 34, Profiles of Drug Substances, Excipients and Related Methodology. 2008. 265–298 p.

78. Chen KL, Liu LC, Chen WR. Adsorption of sulfamethoxazole and sulfapyridine antibiotics in high organic content soils. Environ Pollut [Internet]. 2017;231:1163–71. Available from: http://dx.doi.org/10.1016/j.envpol.2017.08.011

79. Zhu T ting, Su Z xian, Lai W xia, Zhang Y bin, Liu Y wen. Insights into the fate and removal of antibiotics and antibiotic resistance genes using biological wastewater treatment technology. Sci Total Environ [Internet]. 2021;776:145906. Available from: https://doi.org/10.1016/j.scitotenv.2021.145906

80. Mehrtens A, Licha T, Burke V. Occurrence, effects and behaviour of the antibiotic lincomycin in the agricultural and aquatic environment – A review. Sci Total Environ [Internet]. 2021;778:146306. Available from: https://www.sciencedirect.com/science/article/pii/S0048969721013747

81. Carucci A, Cappai G, Piredda M. Biodegradability and toxicity of pharmaceuticals in biological wastewater treatment plants. J Environ Sci Heal - Part A Toxic/Hazardous Subst Environ Eng. 2006;41(9):1831–42.

82. Liu Q, Li M, Liu X, Zhang Q, Liu R, Wang Z, et al. Removal of sulfamethoxazole and trimethoprim from reclaimed water and the biodegradation mechanism. Front Environ Sci Eng. 2018;12(6).

83. Masters PA, O'Bryan TA, Zurlo J, Miller DQ, Joshi N. Trimethoprim-Sulfamethoxazole Revisited. Arch Intern Med [Internet]. 2003 Feb 24;163(4):402. Available from:

https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/215162#:~:text=Thus%2C available preparations are manufactured,in oral and intravenous preparations.

84. Wolter DJ, Scott A, Armbruster CR, Whittington D, Edgar JS, Qin X, et al. Repeated isolation of an antibiotic-dependent and temperature-sensitive mutant of Pseudomonas aeruginosa from a cystic fibrosis patient. J Antimicrob Chemother. 2021;76(3):616–25.

85. Thiebault T. Sulfamethoxazole/Trimethoprim ratio as a new marker in raw wastewaters: A critical review. Sci Total Environ. 2020;715.

86. Archer E, Petrie B, Kasprzyk-Hordern B, Wolfaardt GM. The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. Chemosphere [Internet]. 2017 May;174:437–46. Available from: http://dx.doi.org/10.1016/j.chemosphere.2017.01.101

87. Elshikh MS, Hussein DS, Al-khattaf FS, Rasheed El-Naggar RA, Almaary KS. Diclofenac removal from the wastewater using activated sludge and analysis of multidrug resistant bacteria from the sludge. Environ Res [Internet]. 2022;208:112723. Available from: https://www.sciencedirect.com/science/article/pii/S0013935122000500

88. Kraigher B, Mandic-Mulec I. Influence of diclofenac on activated sludge bacterial communities in fed-batch reactors. Food Technol Biotechnol. 2020;58(4):402–10.

89. Vieno N, Tuhkanen T, Kronberg L. Elimination of pharmaceuticals in sewage treatment plants in Finland. Water Res. 2007;41(5):1001–12.

90. Białk-Bielińska A, Maszkowska J, Mrozik W, Bielawska A, Kołodziejska M, Palavinskas R, et al. Sulfadimethoxine and sulfaguanidine: Their sorption potential on natural soils. Chemosphere. 2012;86(10):1059–65.

91. Doretto KM, Peruchi LM, Rath S. Sorption and desorption of sulfadimethoxine, sulfaquinoxaline and sulfamethazine antimicrobials in Brazilian soils. Sci Total Environ [Internet]. 2014;476–477:406–14. Available from: http://dx.doi.org/10.1016/j.scitotenv.2014.01.024

92. Franklin AM, Williams C, Andrews DM, Watson JE. Sorption and desorption behavior of four antibiotics at concentrations simulating wastewater reuse in agricultural and forested soils. Chemosphere [Internet]. 2022;289(May 2021):133038. Available from: https://doi.org/10.1016/j.chemosphere.2021.133038

93. Fick J, Söderström H, Lindberg RH, Phan C, Tysklind M, Larsson DGJ. Pharmaceuticals and Personal Care Products in the Environment CONTAMINATION OF SURFACE, GROUND, AND DRINKING WATER FROM PHARMACEUTICAL PRODUCTION. Environ Toxicol Chem. 2009;28(12):2522–7.

94. Koba O, Golovko O, Kodešová R, Fér M, Grabic R. Antibiotics degradation in soil: A case of clindamycin, trimethoprim, sulfamethoxazole and their transformation products. Environ Pollut. 2017;220:1251–63.

95. Wang Y, Wang X, Li M, Dong J, Sun C, Chen G. Removal of pharmaceutical and personal care products (PPCPs) from municipalwaste water with integrated membrane systems, MBR-RO/NF. Int J Environ Res Public Health. 2018;15(2).

96. Calcio Gaudino E, Canova E, Liu P, Wu Z, Cravotto G. Degradation of antibiotics in wastewater: New advances in cavitational treatments. Molecules. 2021;26(3).

97. de Ilurdoz MS, Sadhwani JJ, Reboso JV. Antibiotic removal processes from water & wastewater for the protection of the aquatic environment - a review. J Water Process Eng [Internet]. 2022;45:102474. Available from: https://doi.org/10.1016/j.jwpe.2021.102474

98. Arya V, Philip L, Murty Bhallamudi S. Performance of suspended and attached growth bioreactors for the removal of cationic and anionic pharmaceuticals. Chem Eng J [Internet]. 2016;284:1295–307. Available from: http://dx.doi.org/10.1016/j.cej.2015.09.070

99. Hassan M, Zhu G, Lu YZ, Al-Falahi AH, Lu Y, Huang S, et al. Removal of antibiotics from wastewater and its problematic effects on microbial communities by bioelectrochemical technology: Current knowledge and future perspectives. Environ Eng Res. 2021;26(1):1–15.

100. Hoff R, Vogelmann ES, Zapelini de Melo AP, Deolindo CTP, Medeiros BM de S, Daguer H. Reacqua: A low-cost solar still system for the removal of antibiotics from contaminated effluents. J Environ Chem Eng [Internet]. 2021;9(6):106488. Available from: https://www.sciencedirect.com/science/article/pii/S2213343721014652

101. Booth A, Aga DS, Wester AL. Retrospective analysis of the global antibiotic residues that exceed the predicted no effect concentration for antimicrobial resistance in various environmental matrices. Environ Int [Internet]. 2020;141(May):105796. Available from: https://doi.org/10.1016/j.envint.2020.105796

102. Hanna N, Purohit M, Diwan V, Chandran SP, Riggi E, Parashar V, et al. Monitoring of Water Quality, Antibiotic Residues, and Antibiotic-Resistant Escherichia coli in the Kshipra River in India over a 3-Year Period. Int J Environ Res Public Health [Internet]. 2020 Oct 22;17(21):7706. Available from: https://www.mdpi.com/1660-4601/17/21/7706

103. Zhang Y, Gu AZ, Cen T, Li X, He M, Li D, et al. Sub-inhibitory concentrations of heavy metals facilitate the horizontal transfer of plasmid-mediated antibiotic resistance genes in water environment. Environ Pollut [Internet]. 2018;237:74–82. Available from: https://www.sciencedirect.com/science/article/pii/S0269749117343592

104. Gullberg E, Albrecht LM, Karlsson C, Sandegren L, Andersson DI. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. MBio. 2014;5(5).

105. Janz SD, Sherwin GHMAL, York SN, Berlin H. Clinical Pharmacology of Anti-Epileptic Drugs.

106. National Center for Biotechnology Information. PubChem Compound Summary for CID 3033, Diclofenac [Internet]. Vol. 1. 2005. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/3033

107. Emea. COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE - Diclofenac - Bovine milk. Commitee Med Prod Vet use. 2009;(582):1–6.

108. Alestig K. The pharmacokinetics of oral quinolones (norfloxacin, ciprofloxacin, ofloxacin). Scand J Infect Dis Suppl [Internet]. 1990;68:19–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2218417

109. Joint FAO/WHO Expert Committee on, Additives F. Evaluation Of Certain Veterinary Drug Residues In Food. Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO technical report series No. 93. World Health Organization, Singapore. 2006;1–88.

110. Boona JH, Degenb M. Disposition of flumequine in plasma of European eel (Anguillu anguilld) after a single intramuscular injection. 99:213–23.

111. Patel A. Bacterial pyoderma. Consult feline Intern Med. 2006;5:251–9.

112. Giguère S. Antimicrobial Therapy in Veterinary Medicine. 2013;199–210.

113. Corea N. Sulfasalazine. In: Enna SJ, Bylund DBBTTCPR, editors. New York: Elsevier; 2007. p. 1–5. Available from: https://www.sciencedirect.com/science/article/pii/B9780080552323626960

114. Levine APP. The Effect of Sulfapyridine on Experimental Avian Coccidiosis Published by: Allen Press on behalf of American Society of Parasitologists Stable URL: http://www.jstor.org/stable/3272213. 2015;26(3):233–5.

115. Campbell WC. History of the discovery of sulfaquinoxaline as a coccidiostat. J Parasitol. 2008;94(4):934–45.

116. Cuckler AC, Ott WH. Tolerance Studies on Sulfaquinoxaline in Poultry. Poult Sci [Internet]. 1955;34(4):867–79. Available from: http://dx.doi.org/10.3382/ps.0340867

117. Preusch PC, Hazelett SE, Lemasters KK. Sulfaquinoxaline inhibition of vitamin K epoxide and quinone reductase. Arch Biochem Biophys. 1989 Feb;269(1):18–24.

118. Qiu J, Zhao T, Liu Q, He J. Residual veterinary antibiotics in pig excreta after oral administration of sulfonamides. Environ Geochem Health. 2015;

119. Coleman MD, Mihaly GW, Edwards G, Ward SA, Howells RE, Breckenridge AM. Pyrimethamine pharmacokinetics and its tissue localization in mice: effect of dose size. J Pharm Pharmacol. 1985;37(3):170–4.

120. Rifkind D, Freeman GL. 7 - CHEMOTHERAPEUTIC AGENTS. In: Rifkind D, Freeman GLBT-TNPWD in ID, editors. London: Academic Press; 2005. p. 51–4. Available from: https://www.sciencedirect.com/science/article/pii/B9780123693532500126

Appendices

Appendix A

IRB approval

An-Najah National University
Faculty of Medicine & Health Sciences
Institutional Review Board

جامعة النجاح الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref: Mas. April. 2022/12

IRB Approval Letter

Title of Research:

Assessing the prevalence of antibiotic residue in Waste Water Treatment Plants and drinking water in North Palestine: Risk assessment for human health and resistance development

Submitted by :
Ala' Jadoo

Supervisor:
Souad Belkebir, Saed Khiyat

Approved:
19th April, 2022

Your Study Title "**Assessing the prevalence of antibiotic residue in Waste Water Treatment Plants and drinking water in North Palestine: Risk assessment for human health and resistance development**" reviewed by An-Najah National University IRB committee and was approved on 19th April, 2022.

Hasan Fitian, MD
IRB Committee Chairman

IRB

Nablus - P.O Box :7 or 707 | Tel (970) (09) 2342902/4/7/8/14 | Faximile (970) (09) 2342910) E-mail : IRB@najah.edu

Appendix B

Nablus municipality facilitating task letter

An-Najah
National University
Faculty of Graduate Studies

جامعة
النجـــاح الوطنية
كلية الدراسات العليا

التاريخ: 2022/3/27

سعادة السيد رئيس بلدية نابلس المحترم

الموضوع: تسهيل مهمة الطالبة/ الاء منذر حسن جدوع رقم تسجيل (12053762)
تخصص ماجستير الأمراض المعدية

تحية طيبة وبعد ،،،

الطالبة/ الاء منذر حسن جدوع، رقم تسجيل 12053762، تخصص ماجستير الأمراض المعدية في كلية الدراسات العليا، وهي بصدد اعداد الاطروحة الخاصة بها والتي عنوانها:

الكشف عن وجود المضادات الحيوية في المياه الناتجة عن محطات معالجة مياه الصرف الصحي ومياه الشرب في شمال فلسطين: تحديد العوامل المؤثرة على صحة الانسان وظهور مقاومة المضادات الحيوية.
Assessing the prevalence of antibiotic residue in Waste Water Treatment Plants and drinking water in North Palestine: Risk assessment for human health and resistance development.

يرجى من حضرتكم تسهيل مهمتها بالايعاز لدائرة المياه والصرف الصحي في السماح للطالبة ب أخذ عينات مياه من المياه العادمة الناتجة من منطقة غرب نابلس مباشرة قبل دخولها محطة التنقيه ومعالجه المياه العادمه (west Nablus) wastewater treatment plant.
السماح ب أخذ عينات مياه من المياه العادمة المعالجة الناتجة من محطة التنقيه ومعالجه المياه العادمه (west Nablus wastewater treatment plant) وذلك قبل اخراجها من المحطة الى مجرى وادي زومر.

السماح بتزويدها ب الخصائص التاليه للمياه العادمة والمعالجه للعينات التي يتم جمعها المذكوره سابقاً : (COD, BOD, TP, TN, TSS, pH) عن طريق محطة التنقية ومعالجه المياه العادمة غربي نابلس.
وايضاً السماح باجراء الفحوص التاليه على عينتين سيتم جمعهم من المياه العادمه المعالجه الموجودة في مجرى وادي زومر الموجود في منطقة بلدية عنبتا (COD, BOD, TP, TN, TSS, pH) وذلك حتى تكون جميع الخصائص المذكورة قد تم اجرائها في نفس المختبر لضمان صحة النتائج ودقتها واستخدامها لأغراض علمية بحتة.

علماً بأن البيانات والمعلومات سوف تستخدم لأغراض البحث العلمي واستكمال مشروع البحث فقط.

شاكرين لكم حسن تعاونكم.

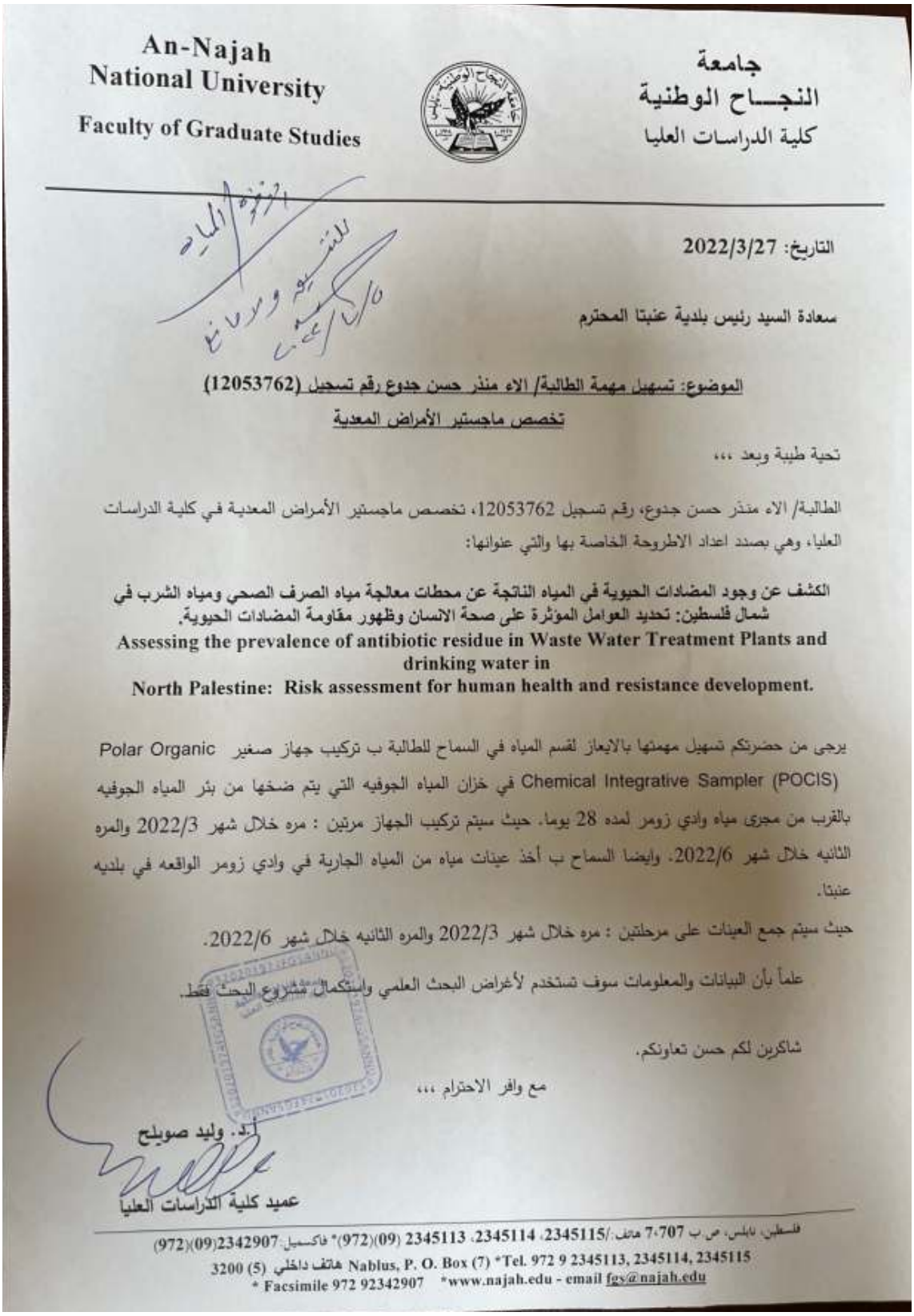
مع وافر الاحترام ،،،

أ.د. وليد صويلح
عميد كلية الدراسات العليا

فلسطين، نابلس، ص.ب 707 هاتف: 2345113-2345114-2345115 (09)(972)* فاكسميل (09)2342907(972)
Palestine, Nablus, P. O. Box (7) *Tel. 972 9 2345113, 2345114, 2345115

Appendix C

Anabta municipality facilitating task letter



An-Najah
National University
Faculty of Graduate Studies

جامعة
النجـــاح الوطنية
كلية الدراسات العليا

التاريخ: 2022/3/27

سعادة السيد رئيس بلدية عنبتا المحترم

الموضوع: تسهيل مهمة الطالبة/ الاء منذر حسن جدوع رقم تسجيل (12053762)
تخصص ماجستير الأمراض المعدية

تحية طيبة وبعد ،،،

الطالبة/ الاء منذر حسن جدوع، رقم تسجيل 12053762، تخصص ماجستير الأمراض المعدية في كلية الدراسات العليا، وهي بصدد اعداد الاطروحة الخاصة بها والتي عنوانها:

الكشف عن وجود المضادات الحيوية في المياه الناتجة عن محطات معالجة مياه الصرف الصحي ومياه الشرب في شمال فلسطين: تحديد العوامل المؤثرة على صحة الانسان وظهور مقاومة المضادات الحيوية.
Assessing the prevalence of antibiotic residue in Waste Water Treatment Plants and drinking water in North Palestine: Risk assessment for human health and resistance development.

يرجى من حضرتكم تسهيل مهمتها بالايعاز لقسم المياه في السماح للطالبة ب تركيب جهاز صغير Polar Organic Chemical Integrative Sampler (POCIS) في خزان المياه الجوفيه التي يتم ضخها من بئر المياه الجوفيه بالقرب من مجرى مياه وادي زومر لمده 28 يوما. حيث سيتم تركيب الجهاز مرتين : مره خلال شهر 2022/3 والمره الثانيه خلال شهر 2022/6. وايضا السماح ب أخذ عينات مياه من المياه الجارية في وادي زومر الواقعه في بلديه عنبتا.

حيث سيتم جمع العينات على مرحلتين : مره خلال شهر 2022/3 والمره الثانيه خلال شهر 2022/6.

علماً بأن البيانات والمعلومات سوف تستخدم لأغراض البحث العلمي واستكمال مشروع البحث فقط.

شاكرين لكم حسن تعاونكم.

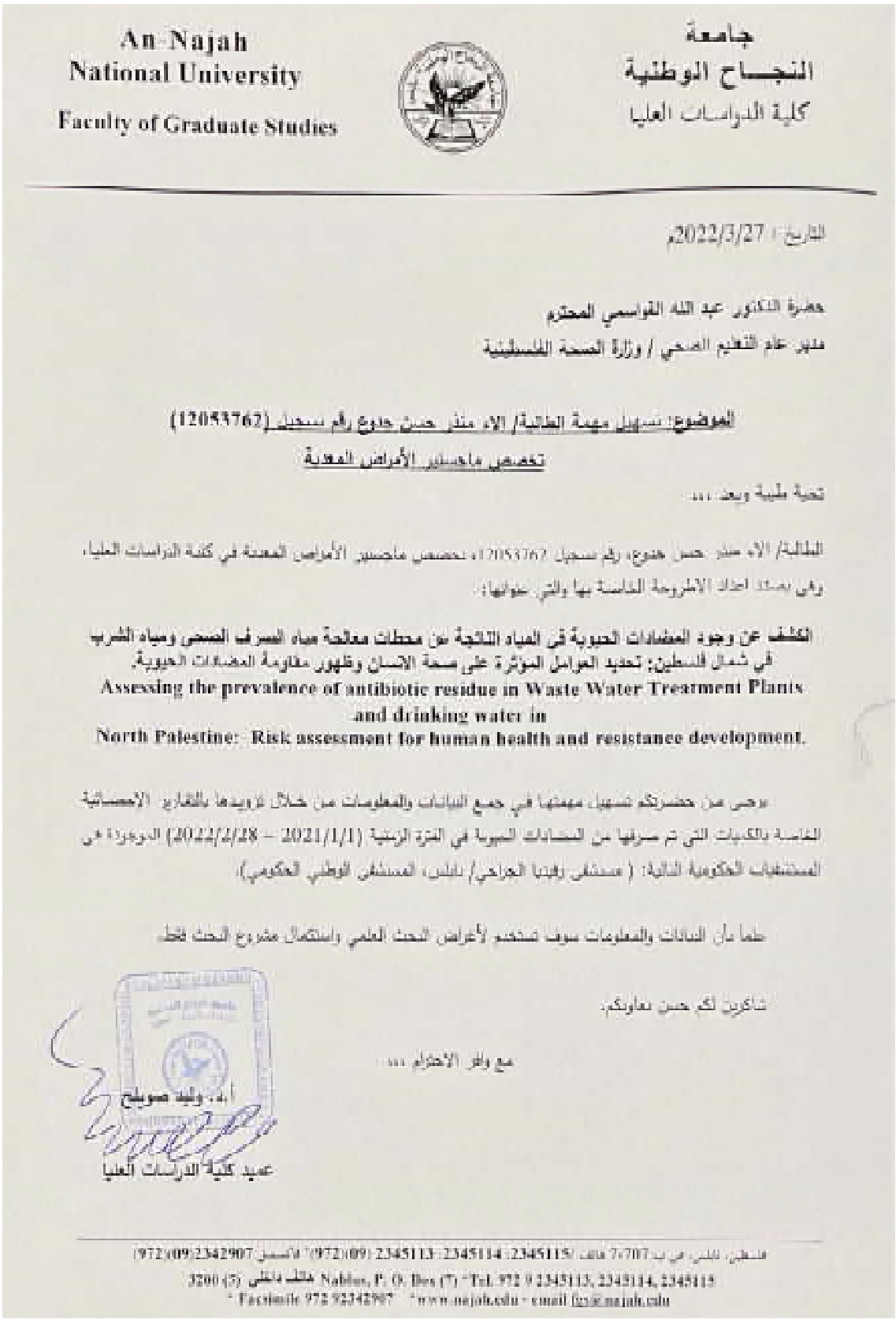
مع وافر الاحترام ،،،

أ.د. وليد صويلح
عميد كلية الدراسات العليا

فلسطين، نابلس، ص.ب 7،707 هاتف: 2345115/، 2345114، 2345113 (09)(972)* فاكسميل 2342907(09)(972)
Nablus, P. O. Box (7) *Tel. 972 9 2345113, 2345114, 2345115 هاتف داخلي 3200 (5)
* Facsimile 972 92342907 *www.najah.edu - email fgs@najah.edu

Appendix D

Palestinian Ministry of Health facilitating task letter



An-Najah National University
Faculty of Graduate Studies

جامعة النجاح الوطنية
كلية الدراسات العليا

التاريخ: 2022/3/27م

حضرة الدكتور عبد الله القواسمي المحترم
مدير عام التقييم الصحي / وزارة الصحة الفلسطينية

الموضوع: تسهيل مهمة الطالبة/ الاء منذر حسن جدوع رقم تسجيل (12053762)
تخصص ماجستير الأمراض المعدية

تحية طيبة وبعد ،،،

الطالبة/ الاء منذر حسن جدوع رقم تسجيل 12053762، تخصص ماجستير الأمراض المعدية في كلية الدراسات العليا، وفي بصدد انجاز الأطروحة الخاصة بها والتي عنوانها:

الكشف عن وجود العضادات الحيوية في المياه الناتجة من محطات معالجة مياه الصرف الصحي ومياه الشرب في شمال فلسطين: تحديد العوامل المؤثرة على صحة الإنسان وظهور مقاومة العضادات الحيوية.
Assessing the prevalence of antibiotic residue in Waste Water Treatment Plants and drinking water in North Palestine: Risk assessment for human health and resistance development.

نرجو من حضرتكم تسهيل مهمتها في جمع البيانات والمعلومات من خلال تزويدها بالتقارير الاحصائية الخاصة بالكميات التي تم صرفها من المضادات الحيوية في الفترة الزمنية (2021/1/1 – 2022/2/28) الموجودة في المستشفيات الحكومية التالية: (مستشفى رفيديا الجراحي/ نابلس، المستشفى الوطني الحكومي).

علما بأن البيانات والمعلومات سوف تستخدم لأغراض البحث العلمي واستكمال مشروع البحث فقط.

شاكرين لكم حسن تعاونكم،

مع وافر الاحترام ،،،

أ.د. وليد مصباح
عميد كلية الدراسات العليا

فلسطين، نابلس، ص.ب 7707 هاتف 2345113 :2345114 :2345115 (972)09 فاكس (972)09 2342907
Palestine, P. O. Box (7) Nablus Tel. 972 9 2345113, 2345114, 2345115 هاتف داخلي (5) 3200
Facsimile 972 92342907 www.najah.edu - email fgs@najah.edu

Appendix E

Palestinian Ministry of Health permission for data collection from two governmental hospitals (Rafedia surgical hospital and Al-Watani hospital)

State of Palestine
Ministry of Health
Education in Health and Scientific
Research Unit

دولة فلسطين
وزارة الصحة
وحدة التعليم الصحي
والبحث العلمي

Ref: .....
Date: .....

الرقم: .....
التاريخ: .....

الأخ مدير عام الإدارة العامة للمستشفيات الحكومية،،،
تحية واحترام،،،

الموضوع: تسهيل مهمة بحث

يرجى التكرم بتسهيل مهمة الطالبة: الآء مفلح حسن قديح – ماجستير أمراض معدية – جامعة النجاح لتعبئة بحث بعنوان:

Assessing the prevalence of antibiotic residue in Waste Water Treatment Plants and drinking water in North Palestine: Risk assessment for human health and resistance development

حيث تتطلب الطالبة جمع معلومات وتقارير احصائية وذلك في:

- مستشفى رفيديا – مستشفى الوطني

مع العلم أن مكان الدراسة: [illegible]

على أن يتم الالتزام بالمحافظة على أخلاقيات البحث العلمي وسرية المعلومات.
على أن يتم الالتزام بجميع التعليمات والإجراءات الوقائية والسلامة المهنية من وزارة الصحة بخصوص جائحة الكورونا، وتحت طائلة المسؤولية، وعلما أن الباحثة ملزمة قبل دخول مرافق وزارة الصحة.
على أن يتم تزويد الوزارة بنسخة PDF من نتائج البحث، التعهد بعدم النشر لحين الحصول على موافقة وزارة الصحة.

مع التحيات،،،

د. [illegible]
رئيس وحدة التعليم الصحي والبحث العلمي

نسخة: عميد كلية الدراسات العليا المحترم/ جامعة النجاح

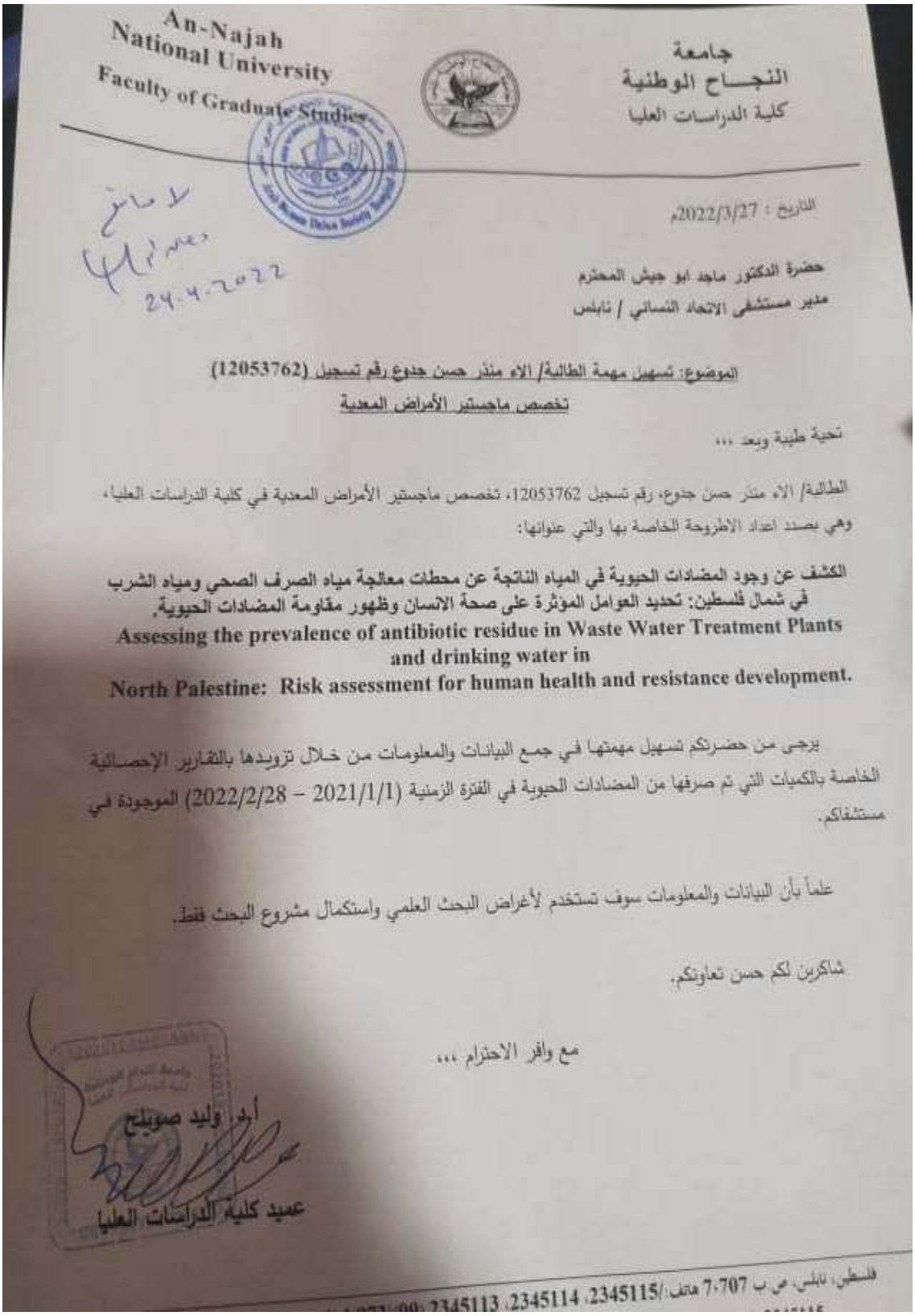
P.O. Box: 14
Tel/fax: 09 2333901

scientificresearch.dep@gmail.com

ص.ب: 14
هاتف: 09 2333901

Appendix F

Al-Itihad hospital facilitating task letter



An-Najah
National University
Faculty of Graduate Studies

جامعة
النجـــاح الوطنية
كلية الدراسات العليا

التاريخ : 2022/3/27م

حضرة الدكتور ماجد ابو جيش المحترم
مدير مستشفى الاتحاد التساني / نابلس

الموضوع: تسهيل مهمة الطالبة/ الاء منذر حسن جدوع رقم تسجيل (12053762)
تخصص ماجستير الأمراض المعدية

تحية طيبة وبعد ...

الطالبة/ الاء منذر حسن جدوع، رقم تسجيل 12053762، تخصص ماجستير الأمراض المعدية في كلية الدراسات العليا، وهي بصدد اعداد الاطروحة الخاصة بها والتي عنوانها:

الكشف عن وجود المضادات الحيوية في المياه الناتجة عن محطات معالجة مياه الصرف الصحي ومياه الشرب في شمال فلسطين: تحديد العوامل المؤثرة على صحة الانسان وظهور مقاومة المضادات الحيوية.
Assessing the prevalence of antibiotic residue in Waste Water Treatment Plants and drinking water in
North Palestine: Risk assessment for human health and resistance development.

يرجى من حضرتكم تسهيل مهمتها في جمع البيانات والمعلومات من خلال تزويدها بالتقارير الإحصائية الخاصة بالكميات التي تم صرفها من المضادات الحيوية في الفترة الزمنية (2021/1/1 – 2022/2/28) الموجودة في مستشفاكم.

علماً بأن البيانات والمعلومات سوف تستخدم لأغراض البحث العلمي واستكمال مشروع البحث فقط.

شاكرين لكم حسن تعاونكم.

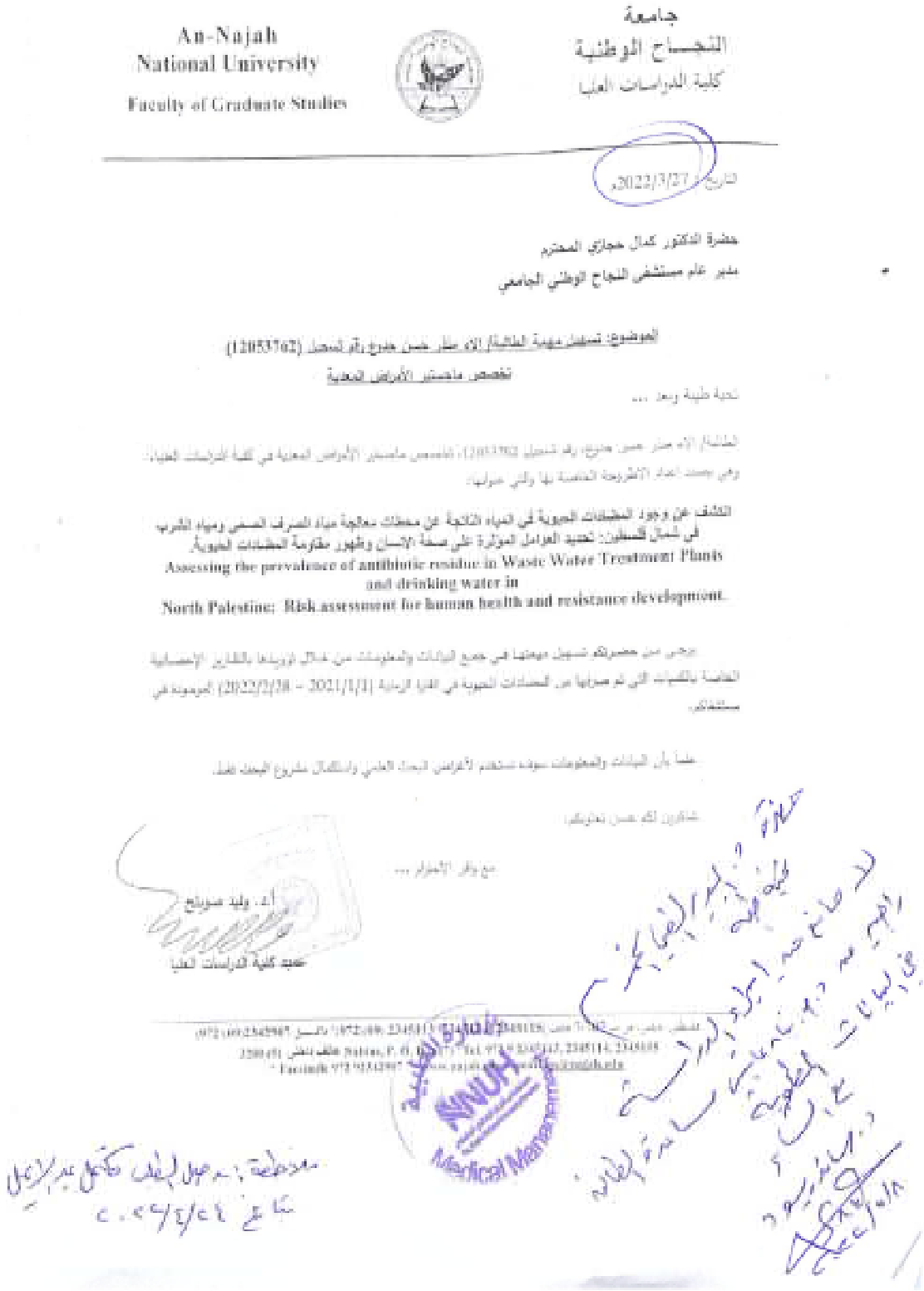
مع وافر الاحترام ...

أ.د. وليد صوبلح
عميد كلية الدراسات العليا

فلسطين، نابلس، ص ب 7707 هاتف: 2345115/ 2345114 2345113

Appendix G

Najah National University Hospital facilitating task letter



جامعة
النجاح الوطنية
كلية الدراسات العليا

An-Najah
National University
Faculty of Graduate Studies

التاريخ 2022/3/27م

حضرة الدكتور كمال حجازي المحترم
مدير عام مستشفى النجاح الوطني الجامعي

الموضوع: تسهيل مهمة الطالبة إلاء منذر حسن عودة رقم الجلوس (12053762)
تخصص ماجستير الأمراض المعدية

تحية طيبة وبعد ...

الطالبة إلاء منذر حسن عودة رقم تسجيل (12053762) تتمم ماجستير الأمراض المعدية في كلية الدراسات العليا، وهي بصدد اعداد الأطروحة الخاصة بها والتي عنوانها:

الكشف عن وجود المضادات الحيوية في المياه الناتجة عن محطات معالجة مياه الصرف الصحي ومياه الشرب في شمال فلسطين: تحديد العوامل المؤثرة على صحة الإنسان وظهور مقاومة المضادات الحيوية
Assessing the prevalence of antibiotic residue in Waste Water Treatment Plants and drinking water in
North Palestine: Risk assessment for human health and resistance development.

نرجو من حضرتكم تسهيل مهمتها في جمع البيانات والمعلومات من خلال توزيعها بالقارئ الإحصائية الخاصة بالكميات التي تم صرفها من المضادات الحيوية في الفترة الزمنية (2021/1/1 - 2022/2/28) المزمعة في مستشفاكم.

علما بأن البيانات والمعلومات ستخدم لأغراض البحث العلمي وللاكمال مشروع البحث فقط.

شاكرين لكم حسن تعاونكم.

مع وافر الاحترام ...

أ.د. وليد صوالحة
عميد كلية الدراسات العليا

هاتف مكتب رئيس جامعة النجاح الوطنية (09) 2345113 / 2345114 / 2345115 فاكس (09) 2345982
P.O. Box 7 Nablus, P. O. Box 7 Tel. 972-9-2345113, 2345114, 2345115
Fax 00972-9-2345982 www.najah.edu

NNUH
Medical Management

Appendix H

List of tested pharmaceuticals in the collected water samples of this study

Pharmaceutical Compounds Tested			
Ampicillin	Erythromycin	Piromidic acid	Sulfamethoxazole
Carbamazepine	Flumequine	Progestrone	Sulfamethoxypyridiazine
Cefaprin	Lincomycin	Pyrimthamine	Sulfamonomethoxine
Cefazolin	Marbofoloxacin	Sarafloxacin	Sulfapyridazine
Ceftiofur	Miloxacin	Spiramycin	Sulfapyridine
Cholramphenicol	Nafcillin	Sulfabenzamide	Sulfaquinoxaline
Chlortetracycline	Nalidixic acid	Sulfabromomrthazine	Sulfathiazole
Ciprofloxacin	Norfloxacin	Sulfacetamide	Sulfatroxazole
Cloxacillin	Ofloxacin	Sulfadiazine	Sulfisomidine
Danofolxacin	Orbifloxacin	Sulfadimethoxine	Sulfisoxazole
Diaveridine	Ormetoprim	Sulfadimidine	Sulfisozole
Diclofenac	Oxacillin	Sulfadoxine	Testosterone
Dicloxacillin	Oxolinic acid	Sulfaethoxypyridazine	Tetracycline
Difloxacin	Oxytetracycline	Sulfaguanidine	Trimethoprim
Enrofloxacin	Penicillin G	Sulfamerazine	Thiamphenicol

جامعة النجاح الوطنية
كليَّة الدراسات العليا

تقييم مدى انتشار المتبقيات الدوائية في منطقة حوض وادي زومر في فلسطين: تقييم مخاطر إعادة الاستخدام وتأثيرها على صحة الانسان

إعداد
آلاء منذر جدوع

إشراف
د. سعاد بلكبير
أ. د. سائد الخياط

قدمت هذه الرسالة استكمالاً لمتطلبات الحصول على درجة الماجستير في مكافحة وضبط العدوى، من كلية الدراسات العليا في جامعة النجاح الوطنية، نابلس–فلسطين.

2023

تقييم مدى انتشار المتبقيات الدوائية في منطقة حوض وادي زومر في فلسطين: تقييم مخاطر إعادة الاستخدام وتأثيرها على صحة الانسان

إعداد

آلاء منذر جدوع

إشراف

د. سعاد بلكبير

أ. د. سائد الخياط

الملخص

ان وجود عدة انواع من المضادات الحيوية والمستحضرات الدوائية في البيئة وعلاقتها السببية في تطور مقاومة مضادات الجراثيم أمر مشكوك فيه حيث أصبحت تعتبر مقاومة مضادات الجراثيم تهديداً صحياً كبيراً. تعتبر المياه المعالجة التي تنتجها محطات معالجة مياه الصرف الصحي المصدر الرئيسي لوجود المركبات الدوائية المختلفة في البيئة بكميات كبيرة.

الهدف من هذه الدراسة الكشف عن مدى وجود المتبقيات الدوائية في انواع مختلفة من المياه مع التركيز على مدى كفاءة محطة معالجة مياه الصرف الصحي في ازالة المستحضرات الدوائية من المياه العادمة.

تم جمع عينتين مياه عادمة لم تتم معالجتها وعينيتين مياه عادمة معالجة وعينتين مياه عادمة معالجة سطحية موجودة في البيئة (وادي زومر–عنبتا) ضمن حملتين منفصلتين. بالاضافة انه تم جمع عينة مياه جوفية باستخدام جهاز أخذ العينات التكاملية الكيميائية العضوية السلبية (Passive Organic Chemical Integrative Sampler, POCIS). تم فحص جميع العينات باستخدام تقنية الاستشراب السائل المزود بمقياس طيف الكتلة (Liquid Chromatography-Mass Spectrometry/ Mass Spectrometry, LC-MS/MS). تم حساب معامل الخطر لقياس مدى خطورة تطور مقاومة مضادات البكتيريا بناءاً على قراءات المضادات الحيوية التي تم قياسها في هذه الدراسة و مقارنتها بالتراكيز المتوقعة لعدم حدوث أي تأثير على البكتيريا.

المستحضرات الدوائية المشتركة بين جميع عينات المياه التي تم جمعها هي: سلفاميثوكزازول (Sulfamethoxazole) و ترايمثوبريم (Trimethoprim)والديكلوفين (Diclofenac) والكارباميزابين (Carbamazepine). كانت كفائة محطة معالجة مياه الصرف الصحي – غرب نابلس عالية في ازالة المستحضرات الدوائية من المياه العادمة في كلتا الحملتين. بالاضافة لذلك كان هناك فرق مكاني كبير في تراكيز المستحضرات الدوائية بين المكان الذي يتم منها تصريف المياه العادمة المعالجة من محطة معالجة مياه الصرف الصحي الى وادي زومر و المكان الذي تم تجميع عينة المياه العادمة السطحية في وادي زومر في قرية عنبتا بعد موسم المطر. التراكيز التي تم الكشف عنها للمضاد الحيوي اوفلوكساسين (Ofloxacin)في عينات المياه العادمة وعينات المياه العادمة المعالجة وعينات المياه العادمة المعالجة السطحية تشكل خطراً لتطور مقاومة مضادات الجراثيم في البيئة. تم الكشف عن تلوث المياه الجوفية بأربع مستحضرات طبية كالتالي: الكاربامیزابين، الديكلوفين، السيبروفلوكساسين والسلفاميثوكزازول.

الوضع الحالي يستدعي القيام بدراسات معمقة لمعرفة مدى الخطر الحقيقي التي تمثله المضادات الحيوية على البيئة ودورها في ظهور وتطور مقاومة مضادات الجراثيم بعد الأخذ بعين الاعتبار المضادات الحيوية التي تم الكشف عنها في هذه الدراسة. التقنيات التي يتم استعمالها في محطات معالجة مياه الصرف الصحي لمعالجة المياه العادمة بحاجة ماسة لتطويرها لزيادة كفاءة المحطة في ازالة المستحضرات الدوائية. تلوث المياه الجوفية يتطلب القيام بدراسات لقياس مدى سمية هذه المركبات على صحة الانسان والحيوان والنبات بشكل مستعجل.

لكلمات المفتاحية: المضادات الحيوية؛ الأدوية؛ مقاومة مضادات الميكروبات؛ مياه الصرف الصحي؛ معالجة مياه الصرف الصحي؛ البيئة؛ الصحة؛ البكتيريا.