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Insecticides in the typical agricultural groundwater in the Gharb plain (Morocco): spatial distribution and health risks

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Abstract
The intensification of agriculture relies on the application of large quantities of phytosanitary products, which contaminate the air, soil, water, and risk human life. This study was carried out in the Gharb plain, which belongs to the Sebou watershed in the North-West of Morocco. The goal of this study is to evaluate the contamination of the groundwater by insecticides using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) analyses. This research paper covers the analysis of 20 samples of 20 different wells for an irrigated perimeter of 250,000 ha of the surface. Further, the analyses show the presence of intense contamination caused by insecticides, which areconcentrated in the southern part of the study area, where the contamination level is 50% of the water samples. This exceeds the norm of the World Health Organization (**WHO)** standard,which is represented in 0.5 µg/l. Furthermore, the analyses also detect the presence of 22 active ingredients of insecticides, such as malathion, methomylalderine, dieldrin, and fenthion. Risk assessment in our studies allowed us to determine that the different RQ calculated for five insecticides (Malathion; Mythomil; chloropyrifos-ethyl; Diazinon) recorded in underground water were up to 1, which proves the intensive use of insecticide in this region and the high risk of contamination.

Keywords
Insecticides; Gharb plain; Morocco; Groundwater; Risk assessment

Introduction

Since the 1950s, the development of agriculture and the desire to increase yields have led to the increasing use of pesticides. These practices have caused, as a result of massive use, contamination of the environment and, in particular diffuse pollution of a large number of aquifers throughout the Moroccan territory. If the focus was primarily on the contamination of the surface network due to high concentrations detected early, the groundwater problem is significant today.

Previously, chemical pesticides, especially insecticides, have been heavily applied to agricultural land (Bidleman and Leone 2004). While most of these pesticides have degraded over time, their effects persist and can be found in the global hydrological cycle, bioaccumulated in the trophic chain, and atmospheric circulation (Wang et al., 2008; Carvalho FP, 2017).

It is now indicated that the pesticide pollution status is worse than historically believed. For instance, some pesticides have been continuously used despite being banned (Hafiane et al., 2021; Zhang et al., 2013). In the past decades, many studies have examined pesticide contaminants in soils, sediments, and ground/surface water, because of their enrichment processes and effects on the ecological systems (Manz et al. 2001; Hu et al. 2009; Bai et al. 2006).

However, studies related to contaminants in groundwater are still in their early stages worldwide, with the systematic regional survey of groundwater pollution in Morocco as an example (Hafiane et al., 2021).

In general, the groundwater survey has focused on a few types of pollutants, such as inorganic compounds, heavy metals, and organic compounds (Marouane et al., 2014), indicating that groundwater in Morocco is very seriously polluted (Elbouzaidi et al., 2020; El Khodrani et al., 2017). As a country with extensive agriculture, the major source of pollution has a considerable influence on groundwater quality in rural areas. The Gharb plain, with its high agricultural potential and high diversity of agricultural practices, knows an intensive use of phytosanitary products in farms, which causes the contamination of the air, soil, and water.

Two different types of processes influence the contamination of the various compartments of the environment by insecticides. The adsorption process binds pesticides to soil particles, where the positive charge of insecticides particle is attached to the negative charge of soil particles, and soils high in organic matter or clay are more adsorptive than coarse and sandy soils (Bouchaib et al., 2007). The insecticide transfer process is sometimes essential to reach the germinating seeds. Where the pesticides can be transferred through volatilization, runoff, leaching, absorption, and crop removal. However, the insecticide can move away from the target pest, which could lead to the contamination of surface water and groundwater (Malhat et al., 2018), where the degradation of insecticides by microorganisms, chemical reactions, and solar radiation can take hours to several years depending on the environmental conditions and the Physico-chemical characteristics of the pesticide (Huang et al., 2018).

These products present high/moderate toxicity and persistence in the environment, causing risks to aquatic organisms and humans. Several cases of contamination in surface and groundwater have been reported worldwide (Palma et al., 2014; Claver et al., 2006; Bortoluzzi et al., 2007; Ribeiro et al., 2014; Milhome et al., 2015; Sousa et al., 2016; Nasr et al., 2020; Abd-Elaty et al., 2021; Farzin et al., 2021; Abd-Elaty et al., 2022).

In addition to the World Health Organization (WHO), several environmental agencies have encouraged the establishment of monitoring programs to assess the quality of water resources to ensure the protection of human health.

The ignorance of risks related to different phytosanitary practices and the absence of pesticide testing and assessment in the region reduces the negative impact on various environmental compartments (Sousa et al., 2016). Including air and water intake, food, and consumer products not achieved, some studies have been conducted in the Gharb region (Nshimiyimana et al., 2014; Berni et al., 2021).

This work aims to assess groundwater contamination level by insecticides in the northwestern part of the Gharb plain based on the quality parameters measurement.

Material and Methods

Study area

The Gharb plain, with an area of 616,000 ha of low altitude, is located in the south of Merja Zerga, 40 km north of the capital Rabat and is stretched over 3 provinces. The plain is formed mainly by Quaternary deposits and delimited by the pre-Rif hills and the southern plateaus, from where three rivers of Sebou, Beht and Ouergha converge into the alluvial plain of Gharb. This plain of Gharb is very flat, the slope being from east to west in all the central and eastern partsand from south to north in the southern part.

The groundwater of this plain is easily recharged by precipitation and irrigation and discharged by high actual evapotranspiration. The plain is characterized by a temperate climate in the coastal zone and a semi-arid climate in the centre(Figure 1).

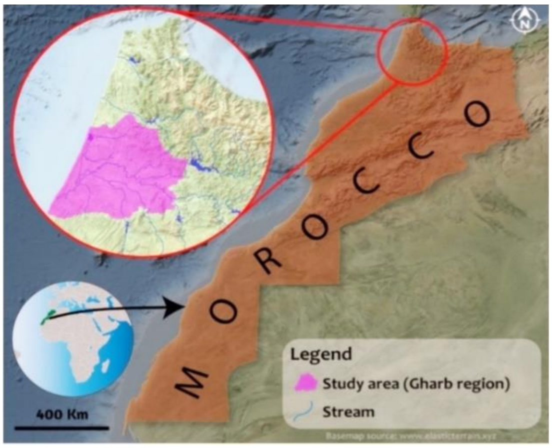

Figure 1. Localization of the study

Geologic and hydrogeologic context

The Gharb perimeter is located in the northwestern part of Morocco. It is surrounded by the Drader-Souier plain to the north, the Maamora plateau to the south, the Atlantic Ocean to the west, and to the east the conglomeratic outcrops that form the boundaries of the basin. With altitudes between 4 and 25 m, it is composed of a coastal zone, continental borders and the central alluvial plain of the Sebou, which is the main wadi. It is one of the most important plioquaternary basins in Morocco, affected by continuous subsidence since the Middle Vindobonian (the period during which the pre-Rif groundwater was laid). Located between two major structural complexes: in the North, the pre-Rif, the Maamora plain and the Hercynian meseta. The pre-Rif domain in the North consists mainly of flysch and paleogenic marls involved in tangential and plicative alpine tectonics with southern vergence or carryings of the front of the Rifaine chain (Mahmouhi et al., 2017).

The primary Meseta in the South (substratum generally formed by schists, quartzites, granites...), constitutes an important province emitting silico-clastic terrigens. These basement soils, strongly folded and intruded with granitoids, regularly sink (3 to 4°) to the north, where they have been reached by drilling, and to the west, these lands are often masked by Cretaceous marl and limestone formations of the Maamora basin. (Hakimi et al., 2019).

Sampling methods

The sampled water points (Figure 2) are selected in such a way as to cover the entire northwestern part of the Gharb plain. In total, twenty samples are subjected to chemical analysis. The groundwater was pumped for 10–15 min before sampling. The Samples were collected in plastic bottles with a minimum capacity of 1liter after being washed with alcohol, deionized water andsoaked with 1:1 HNO_3. Then all samples were immediately chilled at a temperature (of 4C°) and transported by overnight express to the Laboratory for analysis.

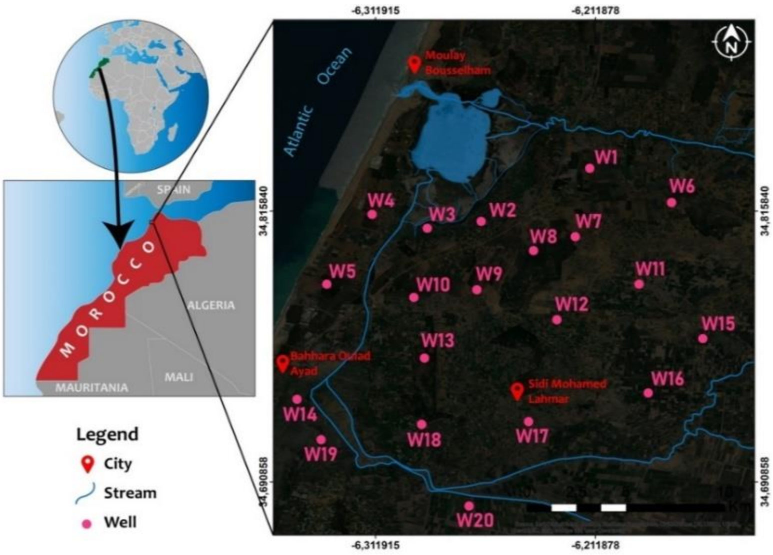

Figure 2. Localization of water samples

Extraction methods
The water samples were processed for pesticide analysis by following the ISO 6468:1996 Méthodes, for the removal of organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes. The water sample (1L) was transferred into a separatory funnel after adding 30 g NaCl. Then, 100 ng of combined surrogate standards mixture (dissolved in acetone) was added. Each sample was extracted several times sequentially under specific pH conditions.

LC-MS/MS and GC-MS/MS analysis. After the extraction, the concentrations of the active ingredients were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and Gas Chromatography - Tandem Mass Spectrometry (GC-MS/MS) techniques wereused to determine the environmental extent of 22 different insecticides (including Abamectine, Aldrine; Bifenazate; Cadusafos; Chlorpyrifos-ethyl; Cyantraniliprole, Cypermethrine; deltamethrine; Endosulfan-sulfate; Dieldrine; Diazinon; ethoprophos; fenazaquine; Fenthion; flubendiamid; imidacloprid; Indoxacarb; Malathion; Methomyl; Parathion-ethyl; Pirimiphos-methyl; Thiamethoxam.) in groundwater samples.

Health risk assessment
Health risk assessment is used in our study to predict the effects of pollutants on human beings over a specified period. Individuals can be exposed to insecticides through several pathways, but oral exposure is considered the most important.

The health risks of insecticides for groundwater in Gharb plain were assessed using the reference doses (RfD) methods (Thomatou et al., 2013).

The ecological pesticide risk can be calculated by using the risk quotient (RQ).

$$RQ = MEC/PNEC \quad (1)$$

A risk quotient (RQ) is the ratio of a point estimate of exposure and a point estimate of effects. It is primarily used to assess the ecological risk of pesticides.

$$RQ = EXPOSURE\ /\ TOXICITY \quad (2)$$

In the above equation, exposure refers to estimated environmental concentration (EEC).

Toxicity refers to an effective level or endpoint obtained from eco-toxicity testing, such as an LC50 or NOEC.The No-observed effect concentration values (NOEC) were used for the calculation of the Predicted no-effect concentration (PNEC). In the case that no NOEC values were available, LC50 or EC50 values were used instead.

PNEC values were calculated by dividing the NOEC (or LC50/EC50) values by the appropriate assessment factor. In particular, when at least one short-term assay (LC50/EC50) at one trophic level was available, an assessment factor of 1000 was applied.

$$PNEC=EC50/AF \quad (3)$$

The individual results of each sample analysis with the maximum concentrations obtained from the monitoring data realized were used as the measured environmental concentration (MEC).

$$MEC=Cs/(Koc*foc) \quad (4)$$

Koc = 1.724 Kom; Kom is the partition coefficient normalized to organic matter,
Koc = Kp/foc,
Kp = soil-water partition coefficient,
Cs isthe concentration of insecticide,
AF is Assessment Factor.

Statistical analysis
The geological and localization mapping were conducted in ArcGis v10.8. Statistical analyses were conducted by excel stat, and Rstudio Statistical software v 4.1.2 wasused for the typological one. The RQmax was determined by the heat map package (R studio) with Ward's agglomeration method and Euclidean distance matrices.

Results

The results show varied concentration values between 0 and 6.71 µg /l, where a minimum concentration of 0 µg/l is recorded at the level of wells P1, P3, P5, P10, P13, P14, P18 and a maximum concentration of 6.71 µg/l at the level of the well P12. The concentration at the level of wells P17, P20 and P4 are 6.69 µg/l, 4.13 µg/l and 4.26 µg/l respectively, which present very high contamination, while the remaining 9 wells contain concentrations between 0.21 µg/l and 3.47 µg/l (Figure 3) (Nshimiyimana et al., 2014).

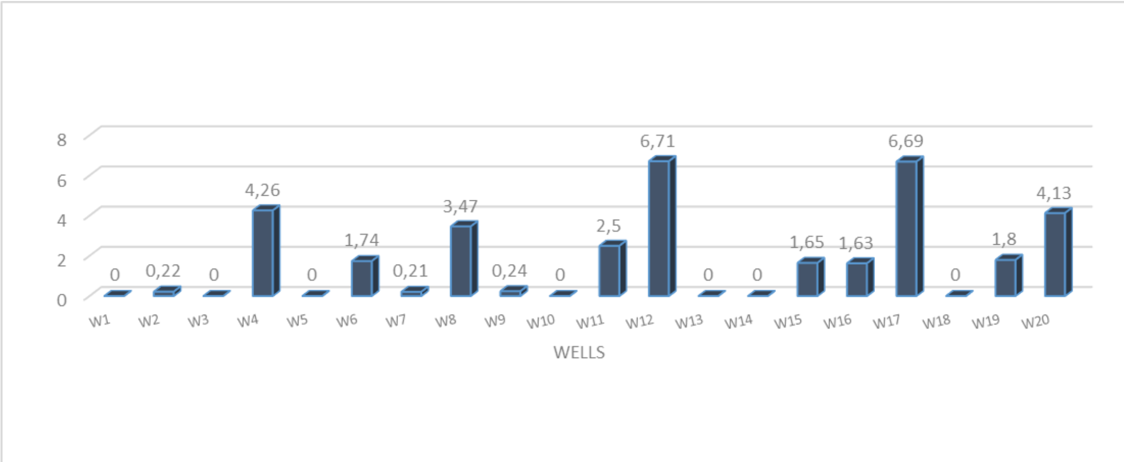

Figure 3. Insecticides concentration in water samples (µg/l)

Figure.4 shows a distribution of wells according to the value of active materials in groundwater. Where some areas know a high concentration and others know a low or an absence of contamination.

The study area is divided into 3 zones (Figure 4). The first zone contains 50% of the wells, where the analysis of 70% of it does not show any concentration of pesticide residues, while the analysis of 30% of wells shows a varied concentration of insecticides between 0.21 µg/l and 0.24 µg/l. Zone 2 contains 20% of wells (P6, P15, P16, P19) with varied concentrations of insecticides between 1.63 µg/l and 1.8 µg/l. Zone 3 contains 30% of wells, where the analyzesshow high contamination, especially in the southern part and in the extreme West (P4, P8, P11, P12, P17, P20), with concentrations of insecticides between 2.5 µg/l and 6.71 µg/l.

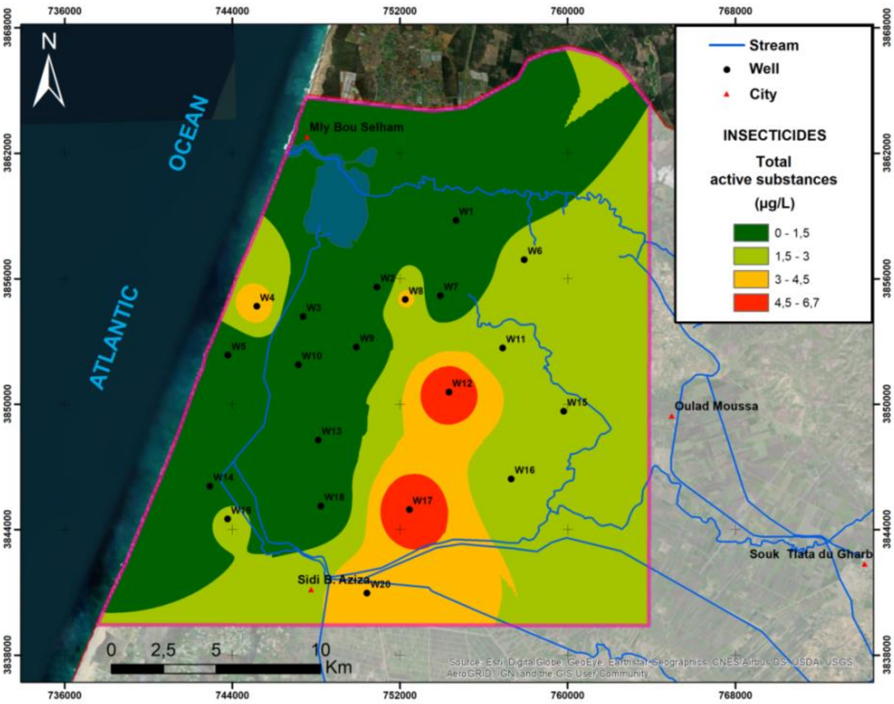

Figure 4. Spatial dispersion of insecticide concentrations.

The results of the correlation matrix (Figure 5) are presented in 22 lines (insecticides) and 13 colons (wells).

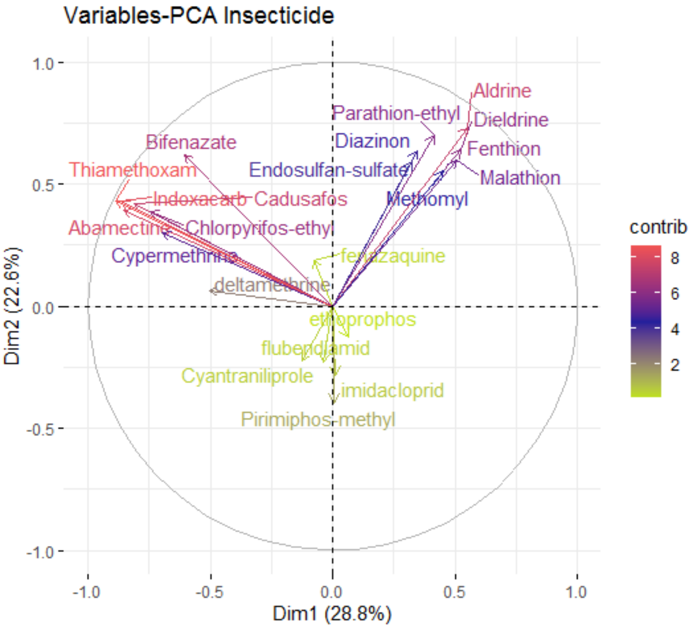

Figure 5. The correlation circle of the active ingredient in insecticides

The results of expressed variance show that the first axis (Dim1) of the correlation circle (Figure 5) extracts 28.76% of the inertia, the Dim2 and Dim3 extract 51.36% and 63.16%, respectively of the inertia, where the 3 axes present more than 73.80% of the total inertia.

Because the first two principal axes reconstruct more than 63.16%, the circle of correlations of the projection plane of the variables Dim1 * Dim2 has been chosen for analysis.

The analysis of the factor map (Figure 6) shows the individualization of 2 groups of samples according to the degree of contamination by the active ingredients belonging to the insecticide class.

The first group is characterized by high levels of active ingredients, including alderine; Parathion-ethyl; Diedrine; diazinon; Endosulfan- sulfate; malathion; methomyl; fenthion, Bifenazate; thiamethoxam; Indoxacarb; Cadusafos; cypermethrin; Chlorpyrifos-ethyl and abamectin. Therefore, wells P20, P17, P12, P4, and P8, which belong to the 1st group, are very contaminated.

The second group is characterized by moderate contamination by cyantraniliprole; ethoprophos; pirimiphos-methyl; flubendiamid, and imidacloprid. Therefore, these wells are less contaminated.

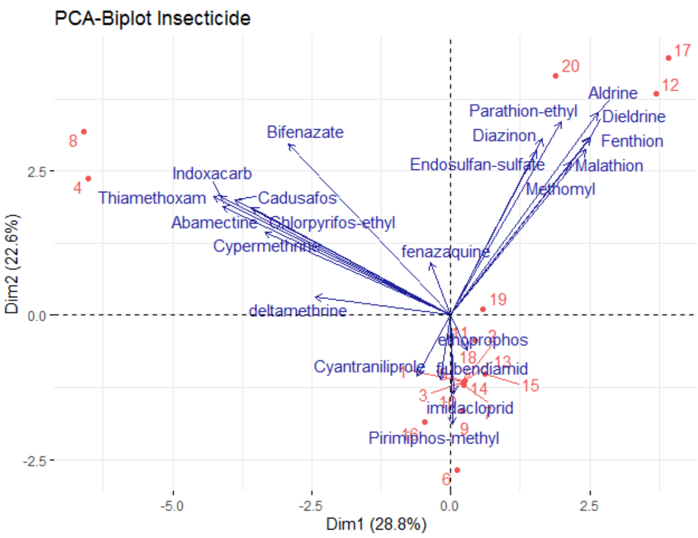

Figure 6. Graphic representation of the factorial analysis of wells

In general, Levels of these pesticide molecules were detected in well water, suggestinga low risk of toxicity. Low risk to very low risk was recorded for all insecticide molecules and at all stations (Figure 7).

A moderate risk of malathion was detected in wells (W19; W17; W12), chloropyrifos-ethyl identifiedin well 8. A moderate to high risk was observed at certain concentrations of active compounds, includingMéthomyl and Diazinon identified in well 20.

The level profile of risk indicates that the pesticide concentrations detected had the chronic toxicity potential for long-term on benthic organisms exposed to groundwater contamination, including the sedentary population (tab.1).

These Results indicated a high positive correlation between the Mode of action of pesticides and the effect of Chemicals Formed When Mixed. Furthermore,another positive correlation between types, soil chemical parameters, the impact of human activities, and doses not respected favours the most risk of chronic toxicity by these molecules.

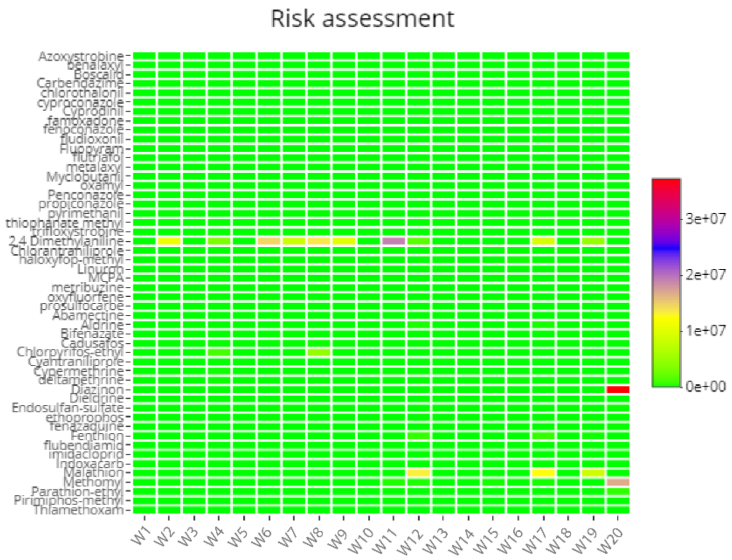

Figure 7: RQ profile of well water in the Gharb area

Tab.1.Data of Risk assessment concentration for each insecticide detected

INSECTICIDE	EC50 [MG/L]	LC50 [MG/L]	KD	LOG KOC	LOG KOW	KOC [MG/L]	FOC	PNEC
ABAMECTINE	6.300	180.000	53.300	4.325	4.400	1.400	38.071	0.006
ALDRINE	0.028	0.006	0.245	6.390	6.500	17.500	0.014	0.000
BIFENAZATE	0.500	0.058	23.100	3.342	3.400	1.778	12.992	0.001
CADUSAFOS	0.001	0.040	4.800	3.785	3.850	0.227	21.145	0.000
CHLORPYRIFOS-ETHYL	0.160	0.001	0.001	4.915	5.000	2.340	0.000	0.000
CYANTRANILIPROLE	0.020	1.200	3.730	1.681	1.710	0.241	15.477	0.000
CYPERMETHRINE	0.000	0.013	54.350	6.822	6.940	30.758	1.767	0.000
DELTAMETHRINE	0.001	0.000	37.900	5.997	6.100	1.024	37.012	0.000
DIAZINON	0.001	0.004	27.100	3.736	3.800	0.609	44.499	0.000
DIELDRINE	0.250	13.300	20.010	5.308	5.400	1.245	16.072	0.000
ENDOSULFAN-SULFATE	150.000	0.074	1500.000	3.559	3.620	0.526	2851.711	0.150
ETHOPROPHOS	0.200	0.320	2.240	2.939	2.990	0.070	32.000	0.000
FENAZAQUINE	0.004	91.000	54.000	5.613	5.710	9.995	5.403	0.000
FENTHION	0.001	15.400	1.830	0.521	0.530	0.018	101.667	0.000
FLUBENDIAMID	0.060	0.060	4.00	4.129	4.200	2.197	2.139	0.000
IMIDACLOPRID	67.200	7.300	4.180	0.561	0.570	0.302	13.841	0.067
INDOXACARB	0.170	0.750	46.300	4.571	4.650	4.483	10.328	0.000
MALATHION	0.000	0.000	1.320	2.320	2.360	1.800	0.733	0.000

METHOMYL	0.008	0.036	0.720	3.185	3.240	0.072	10.000	0.000
PARATHION-ETHYL	0.003	0.000	74.210	3.765	3.830	7.660	9.688	0.000
PIRIMIPHOS-METHYL	0.000	0.056	22.740	4.276	4.350	1.100	20.673	0.000
THIAMETHOXAM	40.000	4.280	11.200	0.895	0.910	0.123	91.057	0.040

Discussion

The analyzes show the presence of aldrin and dieldrin at the level of wells P12, P17, P19, P20 with an average concentration of 0.11 µg / l for aldrin and 0.13 ug / l for dieldrin, which exceed the standard (0.03 µg /l) set by Council Directive 98/83/EC of 3 November 1998. However, previous studies have not detected the presence of aldrin and dieldrin in the groundwater in this region.

The analyzes of samples detect the presence of endosulfan sulfate with a concentration of 0.09 µg / l. According to results found by François Xavier in 2016 (Nshimiyimana et al., 2014), where the concentration of endosulfan sulfate is 0.033µg / l, the concentration of this organochlorine has increased during the last five years but is still considered low according to the standards of European Union (Council Directive 98/83/EC of 3 November 1998), which is similar to studies of insecticides (imidacloprid, chlorpyrifos) were found by Bortoluzzi et al. (2007) in waters (surface and wells) related to the production of tobacco in Rio Grande do Sul, Brazil (Bortoluzzi et al., 2007; Soussa et al., 2016; Milhome et al., 2015).

The water samples studies are characterized by a large variety of pesticides, where we found the samples that contain 10 insecticide types present in the only sample.

The detection threshold (0,1 µg/l) allows the detection of different unauthorized insecticides with variable detection frequencies, such as diazinon detected in Station S5 of Oued Mda and in 15% of wells. Malathion and chlorpyrifos were also found respectively in 15% of well samples and 20 % of groundwater samples.

Jabali Y. and al. determined 80% as the detection frequencyfor methomyl in Libanon in February 2020. This value was high than our results,whichrecorded 40 % (Jabali et al., 2019; Vale et al., 2019).

The Endosulfan was found in the waters of our studied area in wells W12, W17, W19, W20, with an average content of about 0.09 µg/l. In station S5, the content of this substance was 0,96 µg/l. Fekkoul determined the level of endosulfan in water samples collected from Triffa Plain, which varied from 0 to 0,3 µg/l. Xavier et al. (2016) studied the contamination by the same compound of water samples collected from the El Aarjate area (Morroco). This compound was found in relatively low concentrations, 0,033 µg/l. The results of these studies demonstrated that concentrations were lower in Triffa plain and El Aarjate area than the result found in the harb area (Xavier et al., 2016).

The Results issued by Jabali et al. in Lebanon in February 2020 indicate that there were high concentrations of methomyl in water samples collected from surface water (1,5 µg/l) and in the groundwater (0,45 µg/l). These values were higher than those found in our study area, varied from 0,04 µg/l to 0,07 µg/l detected in water samples collected from the water surface, and the average of about 0,22 µg/l as the value detected in water well samples (Jabali et al., 2020).

This active compound, known as Lannate, is too soluble in water and overused by Gharb plain's agriculture. These results were similar to conclusions from other studies (Nassar PP and Ribeiro MG., 2014; Kuharet al., 2015; Wilson M., 2016).

The organochlorine pesticides, specially aldrin and dieldrin, were still detected in some wells W12, W17, W19, W20. Levels of aldrin (0,11 µg/l) and dieldrin (0,13 µg/l). The samples from station S5 contained higher levels of aldrin (0,8 µg/l) and dieldrin (1,36 µg/l), significantly higher than the value required by the WHO (0,03 µg/l) and the standard fixed by Council Directive 98/83/EC on the quality of water intended for human consumption. Moreover,lower than the concentrations found by Chbib in 2020 and Budzinski in 2018 in water samples collected from some wells in Libanon reported respectively 9,33 µg/l and 9,56 µg/l for Aldrin and dieldrin (Budzinski H and Couderchet M. 2018; Chbib et al., 2020).

Other studies, done by authors such as GIROUX in 2010, focus on monitoring residues of water samples collected from 57 wells of the river Châteauguay watershed in (Sud-Ouest de Montréal, Quebec) (Giroux et al., 2010). Low levels of chlorpyrifos have been found in Canada (0,09 µg/l) as compared with the results found in the Gharb area (0,7 µg/l), exactly in the water sample from W8, it was heavily contaminated with this compound.

All pesticides are potentially toxic to all sorts of life, and some are even classified as probable human carcinogens, neurotoxins and endocrine system disruptors (Guo et al., 2014; Malhat et al., 2018). This is proven again by our study of the groundwater in the Gharb plain.

PNEC Values and risk quotient (Rqmax) concern maximum concentration for each insecticide detected higher than LOQ respectively in wells water (Tab.1). The RQmax was higher by 1 for 4 insecticides (Malathion;

Mythomil; chloropyrifos-ethyl; Diazinon); the majorcompounds of insecticides were included in the first list of priority substances of Directive 2013/39/EU.

RQ values were higher for 4 insecticides due to their toxicity for fish and aquatic invertebrates. Risk assessment in our studies made it possible to determine that the different RQ calculated for insecticides (Malathion ; Mythomil ; chloropyrifos-ethyl ; Diazinon) recorded in underground water were up to 1. In general, the concentration levels of the studied compounds were high. This group is used in agriculture inlarge quantities. In consequence, it causes a big ecotoxicological risk to non-target creatures such as aquatic organisms, birds, some beneficial insects and the human population. Vryzas et al. (2009), Papadakis et al. (2015), and Thomatou et al. (2013) also determined the high levels of RQ calculated for insecticides in Evros watershed, Vistonis lake, and Amvrakia lake, respectively.

Conclusions

The results of this study are used to evaluate the chemical contamination of groundwater by insecticides which are dominated by: Malathion; methomyl; alderin; dieldrin, and fenthion. The active substances are found in 65% of wells that are concentrated mainly in the central part of the zone. The existence of these substances is caused by the excessive use of insecticides in the irrigated perimeter of the Gharb, while the strong propagation is explained by the nature of the soil, which is characterized by a sandy loam structure, which facilitates the infiltration into the groundwater. In general, a high rate of contamination by insecticides showed in the groundwater due to phytosanitary practices against pests in farms. Unfortunately, the absence of rigorous actions to preserve and raise awareness among farmers presents a major risk of the deterioration of the environment. Thus, establishing a rational and adapted system that can ensure the monitoring and evaluation of the water quality is necessary.

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