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Changes in Plants and Invertebrate Animals Communities along Gradients of Petroleum Products Pollution

Djaffer Dib*

PSEMRVC Laboratory, Department of Biology, Faculty of Biological Sciences and Agronomical Sciences, Mouloud Mammeri University, Tizi-Ouzou, 15000, Algeria

Djamila Sadoudi Ali Ahmed

PSEMRVC Laboratory, Department of Biology, Faculty of Biological Sciences and Agronomical Sciences, Mouloud Mammeri University, Tizi-Ouzou, 15000, Algeria

*Corresponding author: djaffer.dib@ummto.dz

In this work, we investigated the effects of petroleum product contamination, caused by petrol station wastes, on the vegetation and soil invertebrate animal communities. Three sites situated near two petrol stations were selected, and the vegetation was inventoried during each season of the year. The invertebrates were sampled in the third site using pitfall traps. Results revealed that petrol station wastes negatively affected soil properties (TPH, N, C, pH and moisture). Similarly, plant species and invertebrate communities were influenced in terms of the abundance, species richness, biological diversity (H') and species evenness (J), especially in the most contaminated zones. Some weed plant species showed tolerance to the contamination, as *Xanthium strumarium*, *Avena sp*, *Conium maculatum* and *Oxalis pes-caprae*, and their establishment may have been facilitated by the disturbances caused by the petrol station wastes. Also, some invertebrate orders, as Isopoda and Panpulmonata, were mainly present in the contaminated zones. The high levels of petroleum product contamination and the changes they may cause in soil properties can affect the vegetation and soil invertebrates by influencing the abundance and species richness and by facilitating the establishment of some species, like weed species in the vegetation case.

Keywords: Vegetation, invertebrates, petroleum products, contamination, petrol station.

Introduction

Petroleum products are mixtures composed mainly of hydrocarbons which can cause soil contamination (Henry, 1998; Pinedo, 2013). Some of them are persistent, whilst others are significantly reduced through time by means of many processes (Rhbai et al., 2010), including biodegradation, which depends on many factors (Solano-Serena et al., 2001). Hydrocarbons can cause toxic effects on plant species and soil living organisms when they reach a certain level of concentration (Tang et al., 2011). Hydrocarbon contamination can either positively or negatively affect different environment components, like soil properties (pH, total nitrogen and carbon for example) (Adam and Duncan, 2002; Akpan and Udoh, 2013), germination and growth of plant species (Luhach and Chaudhry, 2012; Fatokun et al., 2015) and macrofauna diversity and abundance (Erstfeld and Snow-Ashbrook, 1999). Transects have already been used to study the effects of pollution gradients on vegetation communities (Dazy et al., 2009) and macrofauna communities (Erstfeld and Snow-Ashbrook, 1999; Grzes', 2009). Ecological analyses can be used to understand changes in vegetation communities (Dazy et al., 2009) and macrofauna communities (Grzes', 2009) along pollution gradients in contaminated sites.

Petroleum products are an indispensable energy source for most countries in the actual context, and their use represents a source of many environmental

contaminations and problems. Thus, the biotechnologies are proposed as alternative green solutions for the restoration of polluted sites (Kathl and Khan, 2011; Mandal et al., 2014). Vegetation sampling through pollution gradients can highlight some petroleum products tolerant plant species, which can be proposed for further research in order to determine their potential for the remediation of contaminated sites.

Petrol stations are potential sources of petroleum product soil contaminations. Even if they are subject to several environmental regulations, there are still significant soil contaminations in the environs of petrol stations, due mainly to fuel spills and fuel tanks leaks (Borowiec et al., 2008; Moschini et al., 2005; Parvizi Mosaed et al., 2015). We know that fuel spills from petrol stations can affect the surrounding environment and people's health (Alharbi et al., 2018; Pearson and Oudijk, 1993); consequently, an investigation about the effects of those petrol station wastes on their surrounding environment will provide some interesting information about the changes they may cause in the soil and the response of living organisms to this situation. In this study, we selected three sites near two petrol stations, and we investigated the effects of the petroleum product contamination on the vegetation and the invertebrate communities, and tried to identify some potential tolerant species to such contaminations.

Materials and Methods

Sites description

This work was performed near two petrol stations, in Freha (36°44,10'40"N, 4°18,24'83"E) and Idjeur (36°39'30.58"N, 4°27'54.51"E), two regions located in the middle-north of Algeria. The climate is Mediterranean continental with cold winters and hot dry summers. Altitudes in Freha and Idjeur are 124 m and 470 m, respectively. Three study sites were selected: the first site is located near the Idjeur petrol station and two other sites near the Freha petrol station. The study sites were

subject to liquid discharges, contaminated with petroleum products emanating from the petrol stations. The wastes are mainly composed of fuels (petrol and diesel), waste oils, lubricating oils and water.

Sampling design

Based on the vegetation structure, it was possible to identify four vegetation gradients that were supposed to be related to the contamination. Then, two transects

(T1 and T2) were established from the rejection point (low vegetation cover) to a distant area supposed to be unpolluted (important vegetation cover), and four zones were delimited in each transect. The first zone is located near the rejection point and the fourth zone is located in the unpolluted zone. This sampling design was used for soil, vegetation and invertebrates sampling (Figure 1 and 2).

Fig. 1. Sampling site 1 (Idjeur petrol station).

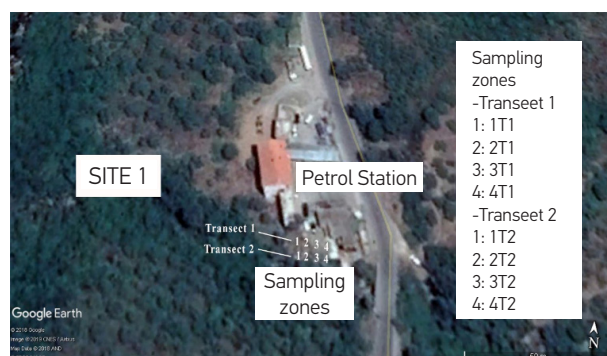
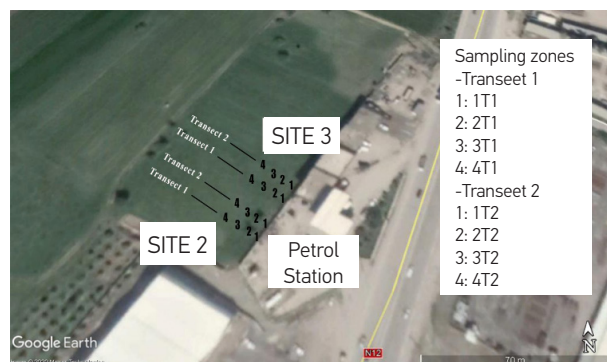


Fig. 2. Sampling site 1 (Idjeur petrol station).



Soil sampling was performed in May 2018. For each zone, soil samples were collected at a depth of 20 cm, put in plastic bags and conserved at 4°C before being analysed. Soil physical-chemical analyses concerned the granulometric composition, pH, electrical conductivity (EC), total Kjeldahl nitrogen (N), carbon (C) and moisture. These analyses were performed at the National Institute of Forester Research (INRF) based in Algiers. Total petroleum hydrocarbons (TPH) analyses were performed at the laboratory of petrochemical

synthesis, in the Faculty of Hydrocarbons and Chemistry (Boumerdes University), using the Mid-IR laser spectroscopy method. TPH analyses were only performed for site 3. Macherey Nagel qualitative oil test paper was used to determine the polluted and the unpolluted zones through the transects of sites 1 and 2.

Vegetation sampling

Four surveys were performed (in November 2017, January 2018, March 2018 and May 2018). For each zone, we delimited a quadrat of 50 X 50 cm, in which we performed the vegetation inventory and the estimation of plant species abundance-dominance developed by Braun-Blanquet et al. (1932). Species richness (S) and abundance (the number of individuals, noted as N), in each zone were estimated. Shannon-Weaver's diversity (H'), Pielou's evenness (J) and Sorensen-Dice coefficient (DSC) were calculated according to Shannon and Weaver (1964), Pielou (1966), Sorensen (1948) and Dice (1945), respectively.

Invertebrates sampling

Soil invertebrates sampling was performed in the third site using pitfall trapping during ten days in August 2018. We used plastic pitfall traps of 9 cm in diameter and 17 cm in height. In each zone of the two transects, one pitfall trap was placed and was third party filled with water, saturated with salt, and a small amount of liquid detergent was added. Pitfall traps were checked every two days (five collections), and trapped individuals were placed in plastic bottles containing 70° alcohol. Invertebrates were identified until the order, and the approximate number of species was determined without the species being identified. Order abundance, number of orders and species richness (S) were estimated for each zone. Shannon-Weaver's diversity (H'), Pielou's evenness (J) and Sorensen-Dice coefficient (DSC) were calculated.

Data analyses

ANOVA followed by the Newman-Keuls test was performed to highlight the significant differences ($p < 0.05$). The Pearson linear correlation method was used to investigate the relationships between soil properties and ecological indexes. All tests were performed under Statistica 7.1 software (©StatSoft).

Results and Discussion

Soil analyses results are represented in Table 1. TPH analyses on site 3 showed a gradual decrease in the contamination through the different zones, from zones 1 (most polluted zones, with 336.35 mg/kg and 333.62 mg/kg) to zones 4 (no pollution detected). Qualitative tests indicated the presence of contaminated zones in sites 1 and 2, and carbon rates in these two sites suggest a clear differentiation between zones 1 and the other zones. For each site, the granulometric composition was fairly similar across the different zones. However, we observed some variations, in particular for sand rates on site 3. Carbon, TPH and moisture rates decreased progressively from zones 1 to zones 4 while total nitrogen showed an opposite tendency. No particular tendency was found for the electrical conductivity. In site 3, the Pearson linear correlation showed a very strong positive correlation ($p < 0.05$) between TPH and C/N ratio (Table 2). The hydrocarbon contamination caused an increase in carbon rates and moisture in all sites, and potentially a decrease in pH values for sites 1 and 2. Similarly, Akpan and Udoh (2013) reported an acidification of the soil and an increase in carbon rates after a contamination by diesel oil. The petrol stations wastes are composed partly of water, which explains the increase in moisture rates. The C/N ratio was altered by the contamination, resulting in an increase of C/N ratios in zones 1 and a decrease when moving to zones 4. It was reported by Adam and Duncan (2003) that changes in the C/N ratio may negatively affect the vegetation development. Parvizi Mosaed et al. (2015) have reported high levels of TPH in the soils of many petrol stations and have observed some effects on physical-chemical properties of the soils. The homogeneous groups (indicated in Table 1 by different letters) highlight the significant differences through the different zones. Zones 1 (most contaminated zones) are often represented in a separate group, in opposition to the other 3 zones, suggesting an important influence of the petrol stations wastes on soil properties in zones 1.

The total species abundances in each zone presented a general significant tendency ($p < 0.05$), showing a progressive increase from zones 1 to zones 4 (Table

3). The unpolluted zones presented the biggest abundances. This tendency is not systematically observed when we look at the species richness, but we can see that zones 1 always present the lowest species richness (Table 3). These observations suggest a negative influence caused by the contamination and the alteration of soil properties. The homogeneous groups (indicated in Table 3 by different letters) highlight the significant differences through the different zones. Zones 1 are often represented in a separate group, in opposition to the other 3 zones, suggesting that the high contamination levels in zone 1 have an important influence on its vegetation.

Abundance-dominance of plant species is represented in Tables 4, 5 and 6. Three species present a good distribution through the different zones in all sites, establishing even in zones 1. Those species are *Avena sp*, *Conium maculatum* and *Oxalis pes-caprae*. However, there are some species that present a good distribution, but they are station specific, as *Elymus repens* in site 3, *Vicia sativa*, *Cerinthe major* and *Senecio vulgaris* in site 1, and *Galactites tomentosa* in site 2. *Xanthium strumarium* showed a preference for zones 1, especially in sites 2 and 3, presenting a tendency to dominate the zones 1; thus, it is rare in zones 3 and absent in zones 4. There are some plant species that avoided the polluted zones, as *Beta vulgaris*, *Senecio vulgaris*, *Malva sylvestris*, *Sonchus oleraceus*, *Galactites tomentosa* and *Scolymus hispanicus*.

Many studies showed the negative effects of hydrocarbon contamination on the growth and germination of plant species, as Fatokun et al. (2015), Adam and Duncan (2002) and Luhach and Chaudhry (2012). Also, these studies revealed that the effects depend on the plant species because not all the species react in the same way to contamination. Likewise, our observations reveal that the contamination affected the species richness, especially in zones 1, the poorest zones in term of species. Some plant species succeeded to establish in the polluted zones, as *Xanthium strumarium*, *Avena sp*, *Conium maculatum* and *Oxalis pes-caprae*. Nevertheless, other species as *Beta vulgaris*, *Senecio vulgaris*, *Malva sylvestris*, *Sonchus oleraceus*,

Table 1. Soil physical-chemical properties in each transect (T1 and T2) of the three sites sampled

Sites	Zones	pH	Clay	Silt	Sand	EC	C	N	C/N	Moisture	TPH
1	1T1	7.6 ^a	28.35	34.82 ^a	36.82	0.42	1.03 ^a	0.03	34.50 ^a	24.43 ^a	Polluted
	1T2	7.9 ^a	29.34	35.45 ^a	35.21	0.4	1.04 ^a	0.03	34.67 ^a	24.53 ^a	Polluted
	2T1	8.2 ^b	36.7	36.33 ^a	26.97	0.34	0.23 ^b	0.05	4.53 ^b	24.35 ^a	Polluted
	2T2	8.2 ^b	41.8	40.18 ^a	18.02	0.43	0.15 ^b	0.04	3.78 ^b	24.36 ^a	Polluted
	3T1	8.4 ^b	39	34.27 ^a	26.73	0.38	0.46 ^c	0.05	9.19 ^c	20.93 ^b	Polluted
	3T2	8.4 ^b	33.45	35.58 ^a	30.97	0.34	0.77 ^c	0.1	7.67 ^c	20.48 ^b	Polluted
	4T1	8.2 ^b	38.5	23.7 ^b	37.81	0.4	0.15 ^b	0.14	1.08 ^d	17.10 ^c	Unpolluted
	4T2	8.2 ^b	22.4	26.8 ^b	52.4	0.32	0.08 ^b	0.1	0.76 ^d	17.48 ^c	Unpolluted
2	1T1	7.5	5.05	43.8 ^a	51.15	0.22	0.95 ^a	0.04 ^a	23.75 ^a	21.34 ^a	Polluted
	1T2	7.4	3.55	43.9 ^a	52.55	0.18	0.92 ^a	0.05 ^a	18.37 ^a	21.08 ^a	Polluted
	2T1	7.4	4.1	31.5 ^b	64.39	0.54	0.38 ^b	0.05 ^a	7.67 ^b	17.25 ^b	Polluted
	2T2	8.5	8.45	30.4 ^b	61.15	0.63	0.46 ^b	0.1 ^a	4.59 ^b	17.33 ^b	Polluted
	3T1	8.1	5.65	31.48 ^b	62.87	0.16	0.77 ^c	0.1 ^a	7.67 ^b	17.16 ^b	Polluted
	3T2	7.9	4.2	31.52 ^b	64.27	0.88	0.69 ^c	0.14 ^a	4.94 ^b	17.03 ^b	Polluted
	4T1	8	9.5	28.64 ^b	61.86	0.88	0.61 ^c	0.25 ^b	2.44 ^b	13.40 ^b	Unpolluted
	4T2	8	2.35	23.01 ^b	74.64	0.89	0.89 ^c	0.24 ^b	3.71 ^b	16.18 ^b	Unpolluted
3	1T1	7.8	3.54	13.96	82.5 ^a	0.48	1.56 ^a	0.05 ^a	31.20 ^a	22.57 ^a	336.35 ^a
	1T2	7.9	2.95	13.15	83.9 ^a	0.48	1.53 ^a	0.05 ^a	30.70 ^a	22.06 ^a	333.62 ^a
	2T1	8.2	8.2	31.9	62.15 ^b	0.41	1.38 ^{ab}	0.1 ^b	13.84 ^b	18.88 ^b	228.34 ^b
	2T2	8	12.5	24.36	63.06 ^b	0.4	1.20 ^{ab}	0.15 ^b	8.00 ^b	19.25 ^b	104.63 ^b
	3T1	7.7	5.35	31.81	62.83 ^b	0.47	0.84 ^{bc}	0.17 ^{bc}	4.96 ^{bc}	17.10 ^c	10.83 ^c
	3T2	7.9	5	31.62	63.37 ^b	0.25	0.92 ^{bc}	0.16 ^{bc}	5.74 ^{bc}	17.12 ^c	28.95 ^c
	4T1	7.9	9.5	26.68	63.82 ^b	0.23	0.69 ^c	0.2 ^c	3.46 ^c	12.80 ^d	0 ^c
	4T2	7.9	4.1	41.93	53.97 ^b	0.42	0.30 ^c	0.25 ^c	1.21 ^c	12.38 ^d	0 ^c

Moisture, C, N, clay, silt and sand results are expressed in percentage, EC in $\mu\text{S}/\text{cm}$ and TPH in mg/kg .

Different letters indicate significant differences between the different zones (ANOVA followed by the Newman-Keuls test, $p < 0.05$)

Table 2. Correlation coefficients between soil physical-chemical properties and ecological indexes

	Site 1				Site 2				Site 3				TPH
	N	S	H'	J	N	S	H'	J	N	S	H'	J	
pH	0.91*	0.78*	0.79*	-0.88*	0.50	0.57	0.64	0.67	-0.09	-0.16	0.05	0.32	0.20
Clay	0.36	0.26	0.29	-0.41	0.09	0.11	0.19	0.30	0.20	0.04	0.20	0.15	-0.32
Silt	-0.33	-0.52	-0.47	0.22	-0.93*	-0.57	-0.72*	-0.82*	0.77*	0.93*	0.88*	0.16	-0.79*
Sand	-0.04	0.13	0.08	0.14	0.89*	0.53	0.65	0.71	-0.79*	-0.88*	-0.86*	-0.16	0.85*
EC	-0.45	-0.33	-0.36	0.36	0.69	0.23	0.42	0.57	-0.54	-0.28	-0.33	0.01	0.59
C	-0.71*	-0.75*	-0.85*	0.54	-0.19	-0.50	-0.61	-0.65	-0.96*	-0.92*	-0.64	0.32	0.90*
N	0.70	0.70	0.63	-0.69	0.87*	0.11	0.26	0.40	0.95*	0.90*	0.68	-0.24	-0.94*
C/N	-0.92*	-0.84*	-0.90*	0.82*	-0.83*	-0.66	-0.79*	-0.83*	-0.85*	-0.88*	-0.84*	-0.08	0.97*
M	-0.71*	-0.82*	-0.75*	0.64	-0.88*	-0.46	-0.61	-0.73*	-1.00	-0.86*	-0.61	0.33	0.88*
TPH	X	X	X	X	X	X	X	X	-0.87*	-0.88*	-0.78*	0.03	1.00

*Significant correlations ($p < 0.05$); M: Moisture

Table 3. Total species abundance (N), species richness (S), Shannon-Weaver diversity (H') and Pielou's evenness (J) indexes in the three sites

Sites	Zones	N				S				H'				J			
		S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
1	1T1	10 ^a	3 ^a	11 ^a	7 ^a	3	1 ^a	5	4 ^a	1.49	0.00 ^a	1.97	1.84 ^a	0.94 ^a	0.00 ^a	0.85 ^a	0.92 ^a
	1T2	8 ^a	4 ^a	12 ^a	7 ^a	3	1 ^a	6	4 ^a	1.50	0.00 ^a	2.13	1.84 ^a	0.95 ^a	0.00 ^a	0.82 ^a	0.92 ^a
	2T1	24 ^b	23 ^b	72 ^b	50 ^b	6	3 ^b	10	7 ^b	2.18	1.40 ^b	2.52	2.43 ^b	0.84 ^{ab}	0.89 ^b	0.76 ^{ab}	0.86 ^b
	2T2	25 ^b	30 ^b	64 ^b	65 ^b	5	4 ^b	9	10 ^b	1.85	1.67 ^b	2.60	2.69 ^b	0.80 ^{ab}	0.83 ^b	0.82 ^{ab}	0.81 ^b
	3T1	31 ^c	31 ^b	89 ^c	77 ^c	5	6 ^c	9	13 ^c	1.57	1.85 ^b	2.39	2.97 ^b	0.68 ^{bc}	0.71 ^b	0.75 ^{ab}	0.80 ^b
	3T2	30 ^c	47 ^b	80 ^c	83 ^c	5	6 ^c	10	10 ^c	1.69	2.05 ^b	2.55	2.50 ^b	0.73 ^{bc}	0.79 ^b	0.77 ^{ab}	0.75 ^b
	4T1	35 ^d	39 ^b	81 ^c	78 ^c	7	9 ^d	8	13 ^d	1.99	2.94 ^c	2.08	2.93 ^b	0.71 ^c	0.93 ^b	0.69 ^b	0.79 ^b
	4T2	36 ^d	38 ^b	86 ^c	76 ^c	5	8 ^d	12	13 ^d	1.40	2.45 ^c	2.62	3.02 ^b	0.60 ^c	0.82 ^b	0.73 ^b	0.82 ^b
2	1T1	17 ^a	1 ^a	12 ^a	19 ^a	4	1 ^a	5 ^a	3 ^a	1.97 ^{ab}	0.00 ^a	2.19	0.95 ^a	0.99 ^a	0.00 ^a	0.94	0.60 ^a
	1T2	15 ^a	1 ^a	8 ^a	14 ^a	4	1 ^a	3 ^a	3 ^a	1.83 ^{ab}	0.00 ^a	1.06	0.73 ^a	0.91 ^a	0.00 ^a	0.67	0.46 ^a
	2T1	40 ^{ab}	72 ^b	38 ^b	47 ^b	5	6 ^b	7 ^b	9 ^b	2.01 ^{ab}	1.43 ^b	1.79	2.56 ^b	0.86 ^b	0.55 ^b	0.64	0.81 ^b
	2T2	31 ^{ab}	57 ^b	30 ^b	40 ^b	7	5 ^b	7 ^b	10 ^b	2.41 ^{ab}	1.33 ^b	2.03	2.82 ^b	0.86 ^b	0.57 ^b	0.72	0.85 ^b
	3T1	53 ^b	33 ^b	51 ^c	61 ^b	6	3 ^c	7 ^b	13 ^c	2.09 ^a	1.45 ^b	1.97	3.09 ^b	0.81 ^b	0.92 ^b	0.70	0.83 ^b
	3T2	35 ^b	66 ^b	46 ^c	52 ^b	7	4 ^c	8 ^b	12 ^c	2.28 ^a	1.09 ^b	2.33	3.03 ^b	0.81 ^b	0.54 ^b	0.78	0.84 ^b
	4T1	55 ^b	78 ^b	56 ^c	73 ^c	6	4 ^c	3 ^a	6 ^d	1.50 ^b	1.23 ^b	1.06	1.99 ^c	0.58 ^c	0.61 ^b	0.67	0.77 ^b
	4T2	55 ^b	78 ^b	57 ^c	85 ^c	6	4 ^c	4 ^a	7 ^d	1.57 ^b	1.23 ^b	1.07	2.19 ^c	0.61 ^c	0.61 ^b	0.54	0.78 ^b
3	1T1	24 ^a	10 ^a	10 ^a	9 ^a	4	4	3	3 ^a	1.87 ^{ab}	1.90	1.49	0.99 ^a	0.94 ^a	0.95 ^a	0.94	0.62 ^a
	1T2	32 ^a	9 ^a	3 ^a	13 ^a	5	3	2	3 ^a	1.98 ^{ab}	1.53	0.92	0.99 ^a	0.85 ^a	0.97 ^a	0.92	0.63 ^a
	2T1	29 ^a	29 ^{ab}	36 ^a	32 ^b	6	6	8	6 ^{ab}	2.05 ^a	2.05	2.04	1.94 ^b	0.79 ^a	0.79 ^b	0.68	0.75 ^b
	2T2	36 ^a	29 ^{ab}	16 ^a	33 ^b	5	7	6	5 ^{ab}	2.04 ^a	2.00	2.15	1.58 ^b	0.88 ^a	0.71 ^b	0.83	0.68 ^b
	3T1	44 ^b	55 ^b	38 ^a	51 ^c	6	5	6	9 ^b	1.62 ^b	1.99	1.85	2.24 ^b	0.62 ^b	0.85 ^a	0.72	0.71 ^b
	3T2	47 ^b	35 ^b	30 ^a	45 ^c	5	3	2	7 ^b	1.58 ^b	1.42	1.00	2.07 ^b	0.68 ^b	0.90 ^a	1.00	0.74 ^b
	4T1	58 ^c	74 ^c	63 ^b	83 ^d	5	4	3	7 ^b	1.47 ^b	1.28	0.82	1.59 ^b	0.63 ^b	0.64 ^c	0.51	0.57 ^a
	4T2	60 ^c	72 ^c	59 ^b	86 ^d	6	4	4	10 ^b	1.74 ^b	1.14	1.05	1.93 ^b	0.67 ^b	0.57 ^c	0.53	0.58 ^a

S1: November sampling; S2: January sampling; S3: March sampling; S4: May sampling

Different letters indicate significant differences between the different zones (ANOVA followed by Newman-Keuls test, $p < 0.05$).

Galactites tomentosa and *Scolymus hispanicus* avoided the contaminated zones. The first lot of species tolerated the contamination and could represent potential candidates for the phytoremediation of hydrocarbon contaminated sites. *Xanthium strumarium*, a plant species which is able to grow in many habitats and conditions (Weaver and Lechowicz, 1983; Saric et al., 2012), preferred zones 1, where the pollution is most important; consequently, this plant species could have a good capacity to establish in hydrocarbon contaminated sites. Besides, Gulezian et al. (2012) reported the tolerance of

Conium maculatum to the metals and PAHs contamination. The second lot of species is potentially susceptible to hydrocarbon contamination. *Xanthium strumarium*, *Avena sp.*, *Conium maculatum* and *Oxalis pes-caprae* are weed plant species, considered in some parts of the world as invasive species (Castro et al., 2007; Nakova et al., 2004; Sharma et al., 2010; Vetter, 2004). Hierro et al. (2006) suggested that human activities could increase species invasions; therefore, it is possible that the changes caused by the contamination helped in the establishment of the weed species.

Table 4. Phytosociological table of site 1 with species abundance-dominance

Zones	November				January				March				Mai																			
	Transect 1		Transect 2		Transect 1		Transect 2		Transect 1		Transect 2		Transect 1		Transect 2																	
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4								
<i>Avena sp</i>	1	2	2	1	1	2	2	1	1	3	3	2	1	3	3	2	+	3	3	3	+	3	3	3	+	3	3	3	1	3	3	3
<i>Ampelodesmos mauritanicus</i>																																
<i>Anogramma leptophylla</i>																																
<i>Daucus carota</i>																																
<i>Centaureum spicatum</i>		+				+								+	+			+														
<i>Cerintho major</i>		+								1	+	1		1	+	1		1	1			1	+			1	+	1		1		
<i>Conium maculatum</i>	1	1	1		1	1	1		2	2	2		1	2	1		1	2	2	2	+	2	2	2	2	2	2		2	2	2	
<i>Foeniculum vulgare</i>																																
<i>Fraxinus angustifolia</i>																																
<i>Galactites tomentosa</i>				1					1	1			2	1								+				1				1		
<i>Geranium robertianum</i>																																
<i>Lathyrus sp</i>																																
<i>Leontodon sp</i>																																
<i>Malva sylvestris</i>				1																												
<i>Medicago sp</i>																																
<i>Oxalis pes-caprae</i>	1	2	2	4	1	1	2	4	1	2	3																					
<i>Plantago lanceolata</i>																																
<i>Rubus fruticosus</i>																																
<i>Senecio vulgaris</i>																																
<i>Sonchus oleraceus</i>																																
<i>Taraxacum sp</i>																																
<i>Trifolium tomentosum</i>																																
<i>Vicia sativa</i>																																
<i>Xanthium strumarium</i>																																

+: range of cover < 5% and few individuals; 1: range of cover < 5% and numerous individuals; 2: range of cover 5–25%; 3: range of cover 25–50%; 4: range of cover 50–75%; 5: range of cover 75–100%.

Table 5. Phytosociological table of site 2 with species abundance-dominance

Zones	November				January				March				Mai																				
	Transect 1		Transect 2		Transect 1		Transect 2		Transect 1		Transect 2		Transect 1		Transect 2																		
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4									
<i>Anagallis moneli</i>													+	+			+	+															
<i>Avena sp</i>	1	2	2	3	1	2	2	3	3	4	4	3	3	4	1	3	4	4	2	4	4	2	2	4	2	2	4						
<i>Beta vulgaris</i>			1	+			+	+			+			+																			
<i>Bromus sp</i>																					+	1	1	2	+	1	1	2					
<i>Bromus sp</i>																									1			1					
<i>Chenopodium album</i>																									2	2		2	2				
<i>Conium maculatum</i>	1	1	1	+	1	1	1	1	1	1		1	1	+	1	+	+	1	+	+	1	1	+	1	1	+	1	1					
<i>Elymus repens</i>																									+			1					
<i>Fumaria capreolata</i>																																	
<i>Galactites tomentosa</i>			+	+		+	+	+		+	+		+	+		+	+	+		+	+	+		+	+	+		+	+	+			
<i>Hordeum murinum</i>																																	
<i>Inula viscosa</i>				+				+	1						1																		
<i>Lamium sp</i>													+			+																	
<i>Lolium perenne</i>																																	
<i>Lysimachia sp</i>								+																									
<i>Malva sylvestris</i>		1	2			1	1										+												1				
<i>Medicago sp</i>																																	
<i>Oxalis pes-caprae</i>	1	2	2	3	+	2	2	3	2	2	3	2	4	3	+	2	3	3	+	2	3	3		1			+	+					
<i>Plantago lanceolata</i>								+																									
<i>Scolymus hispanicus</i>													+			+										+		1	1				
<i>Senecio vulgaris</i>																																	
<i>Sonchus oleraceus</i>		2						+																									
<i>Xanthium strumarium</i>	1					2						+				+		1					1					2	1	+	2	+	+

+: range of cover < 5% and few individuals; 1: range of cover < 5% and numerous individuals; 2: range of cover 5–25%; 3: range of cover 25–50%; 4: range of cover 50–75%; 5: range of cover 75–100%.

Table 6. Phytosociological table of site 3 with species abundance-dominance

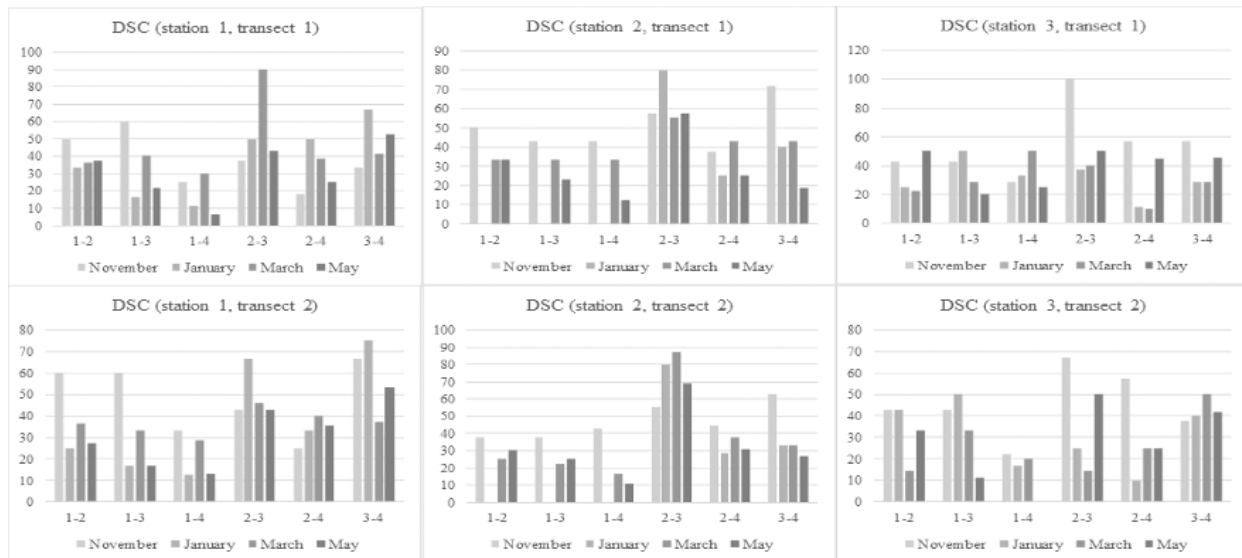
Zones	November				January				March				Mai																		
	Transect 1		Transect 2		Transect 1		Transect 2		Transect 1		Transect 2		Transect 1		Transect 2																
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4							
<i>Anagallis moneli</i>								1					1							+											
<i>Andryala integrifolia</i>																								+							
<i>Avena sp</i>	2	1	2	3	2	2	2	3	1	2	3	4	+	2	3	4	1	1	3	5	+	2	5	+	+	3	3	+	3	3	3
<i>Beta vulgaris</i>				+				1				+				+															
<i>Bromus sp</i>																					+	+	2	4			+	2	4	4	
<i>Bromus sp</i>																											1			1	
<i>Conium maculatum</i>	1	+	+		1	1	1		1	1	2		1	1	1			+	1			+					+	+			
<i>Echium creticum</i>																														+	
<i>Elymus repens</i>	2	+	+		3	1		2				3				1				1					3	1	1		3	1	1
<i>Fumaria capreolata</i>												+																			
<i>Galactites tomentosa</i>															+					1											
<i>Hordeum murinum</i>																									+	1	1			1	1
<i>Inula viscosa</i>				+																											
<i>Lolium perenne</i>																											+			+	+
<i>Malva sylvestris</i>		+	+	+	1	+	+		+	+		+				+				+				+						+	
<i>Oxalis pes-caprae</i>	1	1	3	3	1	1	4	3	+	4	3		4	3	+	3	2	+	4	2											
<i>Phragmites australis</i>																												+		+	
<i>Plantago lanceolata</i>								+																							
<i>Poa annua</i>															+																
<i>Scolymus hispanicus</i>										2				1													+	+		+	+
<i>Senecio vulgaris</i>											+				+	+															
<i>Sonchus oleraceus</i>		+	+					+		1				1				+	+					+							
<i>Trifolium tomentosum</i>																												+		+	
<i>Xanthium strumarium</i>	2				2				2				2	+			1	1				+	3		2	2	+		2	2	+

+: range of cover < 5% and few individuals; 1: range of cover < 5% and numerous individuals; 2: range of cover 5–25%; 3: range of cover 25–50%; 4: range of cover 50–75%; 5: range of cover 75–100%.

We noticed low H' values in zones 1, corresponding to low abundances and low species richness. Sites 2 and 3 presented low H' values in zones 4, and in zones 3 for site 3 (Table 3), due mainly to the *Avena sp* and *Oxalis pes-caprae* dominance. Low H' values in zones 4 of site 1 are due to the *Oxalis pes-caprae* dominance.

The Sorensen–Dice coefficient showed that the highest similarities were between zones 2 and 3, and zones 3 and 4. The highest differences were between zones 1 and 4, and zones 2 and 4 (Figure 3). Thus, there is a clear differentiation between the more polluted zones and the less polluted and unpolluted zones.

Fig. 3. The Sorensen–Dice coefficient (DSC) values of the vegetation sampling in the three sites (Transects 1 and 2)



Pearson linear correlation (Table 2) revealed that the C/N ratio, moisture and TPH are the parameters influencing mainly the different ecological indexes. For site 1, pH is also an important parameter. The abundance and H' seem to be the most influenced indexes; thus, there are no significant correlations for S in site 2 and for J in site 3. Consequently, the vegetation structure was influenced by the contamination and the changes in soil properties.

Concerning soil invertebrates, we identified 10 invertebrate orders, spread over the four zones (Table 7). Hymenoptera was the most abundant order throughout the different zones, followed by the Coleoptera and Araneae orders. The response of invertebrate species to a soil contamination depends on many factors, like the specific behaviour of each species under the stress caused by the pollution and its ability to tolerate it; hence, there is a differentiation between

the invertebrate communities living in polluted and unpolluted soils (van Straalen, 2004). Therefore, we can differentiate two lots of invertebrates. The first lot is composed of Isopoda, Panpulmonata, Orthoptera, Diptera and Mantodea orders, which are mostly present in the most polluted zones. Hymenoptera abundances did not show a clear tendency; we observed the lowest abundances in zones 1 and the highest in zones 2. It was observed that Isopoda and Hymenoptera can take advantage of high levels of contamination (Faulkner and Lochmiller, 2000), and according to Gospodarek et al. (2019) survival and body mass change of *P. scaber* reared in investigated soils were observed. The delivered food was not contaminated with PDSs. *P. scaber* reveals a considerable resistance in a short (4 weeks, Isopoda could have good capacities for the biotransformation of petroleum-derived substances and a low vulnerability to the penetration

of these substances through the integuments. Panpulmonata are known for their ability of bioaccumulation and are used as bioindicators (Oehlmann and Schulte-Oehlmann, 2003). In their study, García-Segura et al. (2018) reported a decrease of Gastropods populations with an increase in TPH levels. The second lot is composed by Parasitiformes, Hemiptera, Araneae and Coleoptera orders, which showed a preference for the less polluted and unpolluted zones. Rusin and

Gospodarek (2016) observed in their study that petroleum-based substances in most cases did not have a negative effect on the number of spiders; on the other hand, Gospodarek et al. (2019) survival and body mass change of *P. scaber* reared in investigated soils were observed. The delivered food was not contaminated with PDSs. *P. scaber* reveals a considerable resistance in a short (4 weeks) observed a negative correlation between the Acari and some hydrocarbons.

Table 7. Invertebrate order abundance in each zone of each transect and total abundance for the orders

Zones	1T1	1T2	2T1	2T2	3T1	3T2	4T1	4T2	Total
Araneae	13	7	12	12	21	17	21	18	121
Coleoptera	4 ^a	10 ^a	19 ^{ab}	35 ^{ab}	56 ^c	73 ^c	41 ^{bc}	46 ^{bc}	284
Diptera	2	5	6	6	1	4	2	4	30
Hemiptera	3	2	2	8	11	12	5	10	53
Hymenoptera	125	153	347	239	181	178	376	189	1788
Isopoda	3	11	1	4	2	1	1	0	23
Mantodea	0	1	1	0	0	0	0	0	2
Parasitiformes	1	1	2	4	4	5	15	5	37
Orthoptera	0	2	1	1	1	0	0	0	5
Panpulmonata	1	0	4	1	0	0	0	1	7

Different letters indicate significant differences between the different zones (ANOVA followed by the Newman-Keuls test, $p < 0.05$).

Order abundance seems to be influenced by the soil contamination. There is a general tendency, showing a clear differentiation between zones 1 and the other zones. Zones 1 present the lowest abundance values (Table 8). The number of orders were not negatively influenced by the contamination; thus, zones 4 presented the lowest values (Table 8). However, when it comes to the species richness (Figure 4), there is an obvious tendency demonstrating that zones 1 are the poorest zones. H' and J values present a general tendency, showing higher values for zones 3 and 4 compared with zones 1 and 2 (Table 8). Sorensen–Dice coefficient values for transect 1 did not show any tendency, but in transect 2, the lowest value was between zones 1 and zones 4 (Figure 5). Therefore, there is a differentiation between the most polluted zones and the less polluted

and unpolluted zones. Furthermore, there are some other parameters to consider, as the relationships between the vegetation structure and the invertebrates. It was shown that some invertebrate groups like Coleoptera and phytophagous insects have a close relationship with vegetation structure and plant species diversity (Chenchouni et al., 2015). The distribution of some groups, as Coleoptera and Hemiptera individuals could be partly explained by the vegetation cover, which is more important in zones 4, followed by zones 3, 2 and 1, respectively. Araneae distribution could also be related to the Hemiptera distribution, since they are predators of these insects. So, invertebrate communities can be affected directly by the contamination and indirectly by the changes in the vegetation structure produced by the contamination.

Table 8. Total species abundance (N), number of orders (NO), Shannon-Weaver diversity (H') and Pielou's evenness (J) indexes in each zone of each transect

Zones	N	NO	H'	J
1T1	152	8 ^a	1.07	0.36
1T2	192	8 ^a	1.24	0.41
2T1	395	10 ^b	0.82	0.25
2T2	310	9 ^b	1.28	0.40
3T1	277	8 ^a	1.53	0.51
3T2	290	7 ^a	1.57	0.56
4T1	461	7 ^a	1.03	0.37
4T2	273	7 ^a	1.45	0.52

Different letters indicate significant differences between the different zones (ANOVA followed by the Newman-Keuls test, $p < 0.05$).

Fig. 4. Sorensen–Dice coefficient (DSC) values of the invertebrates sampling (Transects 1 and 2)

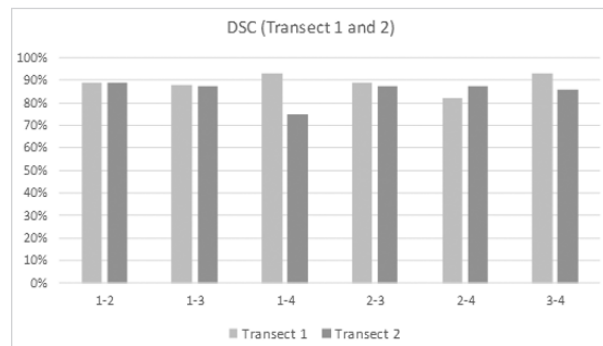
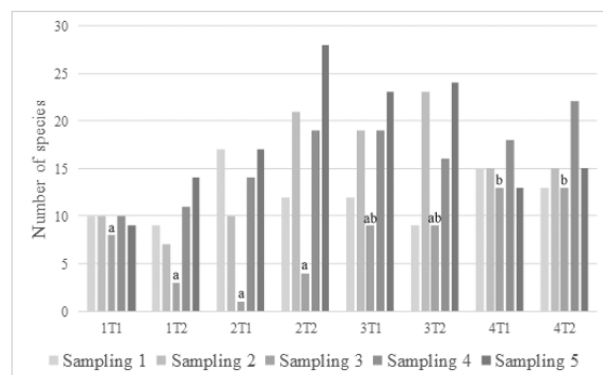


Fig. 5. Invertebrates number of species in each zone of each sampling. Different letters indicate significant differences between the different zones (ANOVA followed by the Newman-Keuls test, $p < 0.05$)



Conclusions

Petrol station wastes affected some soil properties as the TPH, N, C, pH and moisture. The vegetation and soil invertebrates were influenced as regards the abundance, the species richness, the biological diversity (H') and the species evenness (J) by the contamination and the changes in soil properties. Some weed plant species showed a tolerance to the contamination, as *Xanthium strumarium*, *Avena sp*, *Conium maculatum* and *Oxalis pes-caprae*, and some invertebrate orders, as Isopoda, Panpulmonata and Orthoptera were mostly present in the contaminated zones.

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