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EFFECTS OF OLIVE OIL MILL WASTES ON A STREAM MACROINVERTEBRATE COMMUNITY OF CRETE: PRELIMINARY RESULTS

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Introduction

About 60% of the streams of Crete can be characterized as grossly polluted or of doubtful or poor quality. One of the major pollutants, apart from domestic and agricultural wastes, originates from olive oil mills. In Crete today, operate 630 olive oil mills, the highest number of them on the northern part of the island (MICHELAKIS & KOUTSAFTAKIS, 1989).

One kg of olives adds to the environmeent 0.65 lt to 1 lt of wet wastes, the exact quantity depending on the squeezing procedure (pressure or centrifugation) (MICHELAKIS & KOUTSAFTAKIS, 1989). This prodecure includes the following steps (in brief):

- washing of olives
- pressing of olives to get a pulp
- separation of oil from the pulp by pressure or centrifugation.

The water used in all the previous steps plus the pulp which remains after the separation of oil, consistutes the wet wastes.

In Crete every year and only during a three month period - the harvest period, usually December, January and February -, 370,000tn of wet wastes coming from olive oil mills, are disposed to the environment, most of them reaching intermittent streams because the water from permanent streams is usually used for irrigation or drinking and therefore protected. This enormous amount of wastes is fortunately diluted with water to a level of 55-60% into the olive oil mill and during the squeezing procedure and is diluted again when it is disposed into the water of the streams.

The physicochemical characteristics of the wastes are given in Table 1 (GEORGAKAKIS et al., 1986)

TABLE 1. Chemical characteristics of the wastes disposed from the olive oil mill of the Union of Productive Associations in Peza, Crete (GEORGAKAKIS et al, 1986).

TYPE OF ANALYSIS	VALUES***		
Total solids (%)*	6.39	K_2O (mg/l)	6.371
Organic matter (%)**	5.60	Ca (mg/l)	271
C.O.D (mg/l)	99.388	Mg (mg/l)	234
pH	5.21	Fe (mg/l)	154
Conductivity (MS)	9.733	Zn (mg/l)	4.7
$B.O.D_5$ (mg/l)	23.175	Mn (mg/l)	2.3
B.O.D. total (mg/l)	42.738	Cu (mg/l)	1.6
Volatile acids (mg/l)	5.953	Na (mg/l)	296
NH_3-N (mg/l)	47.87	Org.C (%T.sol.)	61.23
NO_3-N (mg/l)	65.50	Total N (%T.sol.)	1.21
P_2O_5 (mg/l)	22.20	C/N	51.7/1

* Drying at 103°C
** Burning at 550°C
*** Mean values of the years 1983-1986.

The wastes entering the streams, form a gel which covers the stream bottom, thus deoxygenating the sediment. These unfavourable conditions together with the toxic substances of the wastes (mainly phenols) are the major threat for the fauna of running waters (HYNES, 1960).

This paper aims to present some first results which are part of a study on the effects of olive oil mill wastes on the benthic macrofauna of three intermittent streams in Crete, differing essentially in the discharge of water. The study covers the period from October 1988 to July 1989, under a monthly interval sampling program.

The data presented here, come from two of the three sampling stations of Prinopotamos, one of the three streams of the study with the lowest discharge of water, and cover a two month sampling period, January and March. The selection of these months was not by chance. January is the month with the highest load of wastes being disposed into the stream, while on March there are no more wastes. A first view of the situation of the benthic macrofauna population under polluted and non polluted conditions, is so achieved.

Description of the study area

Prinopotamos is a small, intermittent stream which carries little flow over the year (from December to March or April). It is a tributary of Aposelemis stream, draining however a different basin. The stream originates from the northeastern part of the village Pigi, (35°14' N, 25° 20' E) and flowing northwest for about 8 km, it reaches Aposelemis stream which then flows into the sea at the north coast of Crete (fig. 1). Stream elevation is 340m at the headwaters and 120m at the mouth.

a.

b.

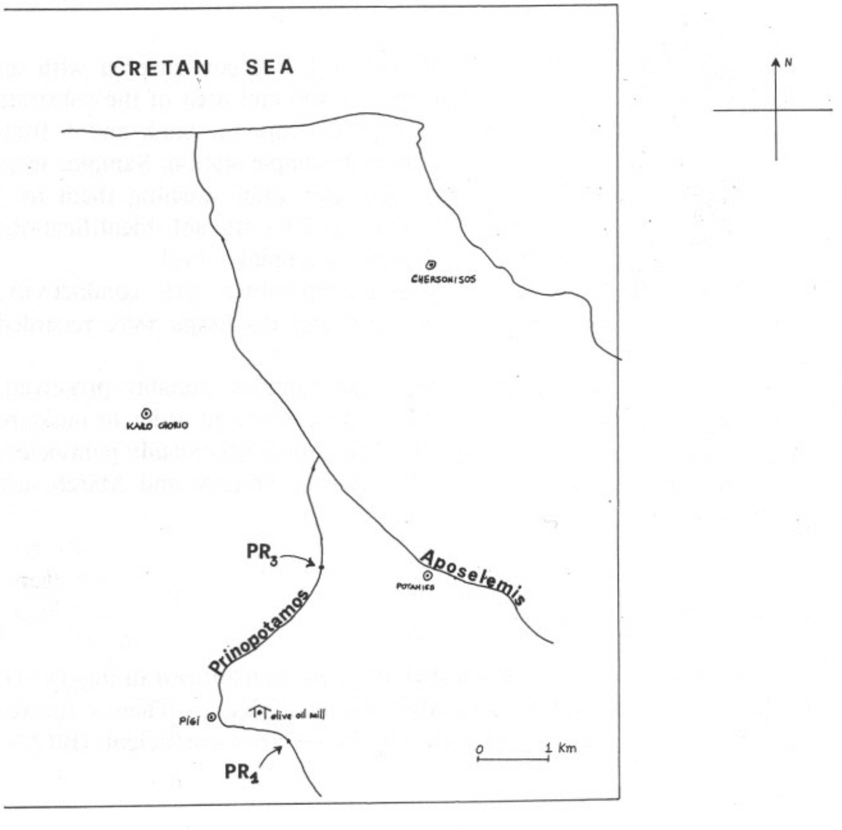

Fig 1. a. Map of the island of Crete showing the area of investigation.
b. Map of Prinopotamos stream showing the sampling stations PR_1, PR_3 and the source of pollution (the olive oil mill).

Stream depth varies from 2-7cm in flats, and 15-25cm in riffles. Width is small near the headwaters (1-1.5 m), increasing to 3-3.5m in the lower sections and reaching 6-8m near the mouth.

Cover along the stream also varies from tree-lined banks to open unshaded areas.

The stream bed consists mainly of limestone. The substrate is coarse with cobbles up to 15cm diameter, pebbles, gravel and sand. Wet wastes from the olive oil mill (the main source of pollution) enter Prinopotamos 2.5 km from the head of the stream. Few domestic wastes enter the stream from the village Pigi.

Sampling station PR_1 is situated in the upper clean part of the stream, 480m before the olive oil mill, thus serving as a control station.

Station PR_3, the polluted station, is located at a distance of 3.5 km after the olive oil mill and about 4 km downstream of station PR_1.

Methods

Benthic invertebrates were collected with a 1 lt scoop covered with an iron net (0.3mm mesh size). For each sample, a 400 cm^2 area of the substrate was disturbed. A total of 10 replicate samples (5 from the bank and 5 from the middle of the channel) were collected at each sample station. Samples were preserved in the field and sorted in the laboratory after washing them in a sieve of 0.5 mm mesh size and preserving them in 70% ethanol. Identifications were carried out to the possible - until now -taxonomic level.

For each sampling and station, water temperature, pH, conductivity, dissolved oxygen, channel width, water depth and discharge were recorded in the field.

In parallel, at each sampling station, water samples, suitably preserved, were taken from the banks and the middle of the channel in order to measure in the laboratory $B.O.D._5$, and C.O.D. The physicochemical parameters measured at sampling stations PR_1 and PR_3 during January and March, are presented in Table 2.

Statistical treatment of results

The raw data of animal species abundance, were transformed to $\log_{10}(x+1)$, to correct for zero values and to normalize the distributions. Then a species similarity matrix was created, based on the Bray-Curtis coefficient (BRAY, 1957). The community diversity was measured using the Shannon-Wiener index (H') (SHANNON & WEAVER, 1949).

TABLE 2. Physical and chemical parameters measured at sampling stations PR_1 and PR_3, during January and March 1989.

sampling stations / parameters	JAN PR_2 bank	middle channel	JAN PR_3 bank	middle channel	MAR PR_1 bank	middle channel	MAR PR_3 bank	middle channel
pH	7.68	7.66	7.30	7.35	7.54	7.43	7.30	7.40
Conductivity (mS)	950	943	716	697	998	1010	760	751
Dissolved Oxygen (mg/l)	10.4	10.2	3	3.2	8.4	8.2	2.2	2.4
Water Temperature (C)	11	11	14	14	13.5	14	15	15
Water Depth (cm)	7	2	16	18	5	4	15	25
Channel Width (m)	1		3		1		2.5	
Discharge (m^3/sec)	0.04		0.07		0.03		0.03	
$B.O.D_5$	0		160		0		22	
C.O.D	0		400		0		55	

The graphical description of faunal relations based on the Bray-Curtis similarity matrix was represented using two multivariate techniques, classification and ordination.

For classification, we used the hierarchical agglomerative clustering based on group averaging of the Bray-Curtis similarity measure (LANCE & WILLIAMS, 1967). The replicates were fused into groups according to their species abundance data.

For ordination, we used the multidimensional scaling representation (MDS) (KRUSKAL, 1977). The significance of differences between some replicates was also tested using an Analysis of Similarity test (ANOSIM). All statistical tests were carried out using the Plymouth Marine Laboratory statistical package.

Results

The composition of the macrobenthos, as numbers of individuals of the main animal groups, is presented in Table 3.

TABLE 3: Mean number of individuals in different animal groups.

x : arithmetic mean of 5 replicate samples
s.d. : standard deviation of the 5 replicate samples

	PR_1 JANUARY 1989				PR_1 MARCH 1989			
	BANK		MIDDLE		BANK		MIDDLE	
	x	s.d	x	s.d.	x	s.d	x	s.d.
Nematoda	90.8	70.7	20.6	6.4	125.6	72.2	35.8	18.5
Annelida	139.2	129.4	146.6	82.8	54.2	24.3	59.2	10.8
Trichoptera	1.6	0.8	0.8	0.4	4.2	1.3	18.2	6.6
Plecoptera	0.2	0.4	8.2	2.7	48.4	18.9	31.0	17.7
Coleoptera	1.0	1.4	0.4	0.5	1.4	2.0	1.4	0.5
Odonata	1.0	1.7	0	0	1.4	0.8	0	0
Ephemeroptera	0.6	0.8	8.0	3.8	30.4	33.3	75.8	53.1
Diptera	71.2	36.1	22.0	6.7	256.2	79.4	128.0	51.7
Ostracoda	11.8	7.0	0.2	0.4	9.2	4.9	8.8	4.0

	PR_3 JANUARY 1989				PR_3 MARCH 1989			
	BANK		MIDDLE		BANK		MIDDLE	
	x	s.d	x	s.d.	x	s.d	x	s.d.
Nematoda	1.4	0.8	1.2	0.8	2.6	1.1	0.2	0.4
Annelida	36.8	26.7	0.8	1.3	216.0	300.5	13.8	11.3
Diptera	24.4	15.6	7.4	5.9	205.4	247.3	60.6	32.9
Ostracoda	1.2	1.0	0.2	0.4	1.2	1.3	0	0
Collembola	8.4	11.4	0.6	0.8	0	0	0	0

The similarities between the two sampling sites PR_1 and PR_3 based on the data of Table 3, produced the dendrogram presented in figure 2. Two clusters, A & B, at approximately 25% similarity level, are prominent. Cluster A is an aggregation of all replicates (1 to 20) from the control site PR_1 for both months, January and March. Cluster B is an aggregation of all replicates (21-40) from the polluted site PR_3 for both months, January and March. There is also a clear separation into four subclusters A_1, A_2, A_3, A_4 from cluster A. A_1 and A_2 are groupings of replicates for January (A_1 a subcluster including replicates from the middle of the channel and A_2 a subcluster including

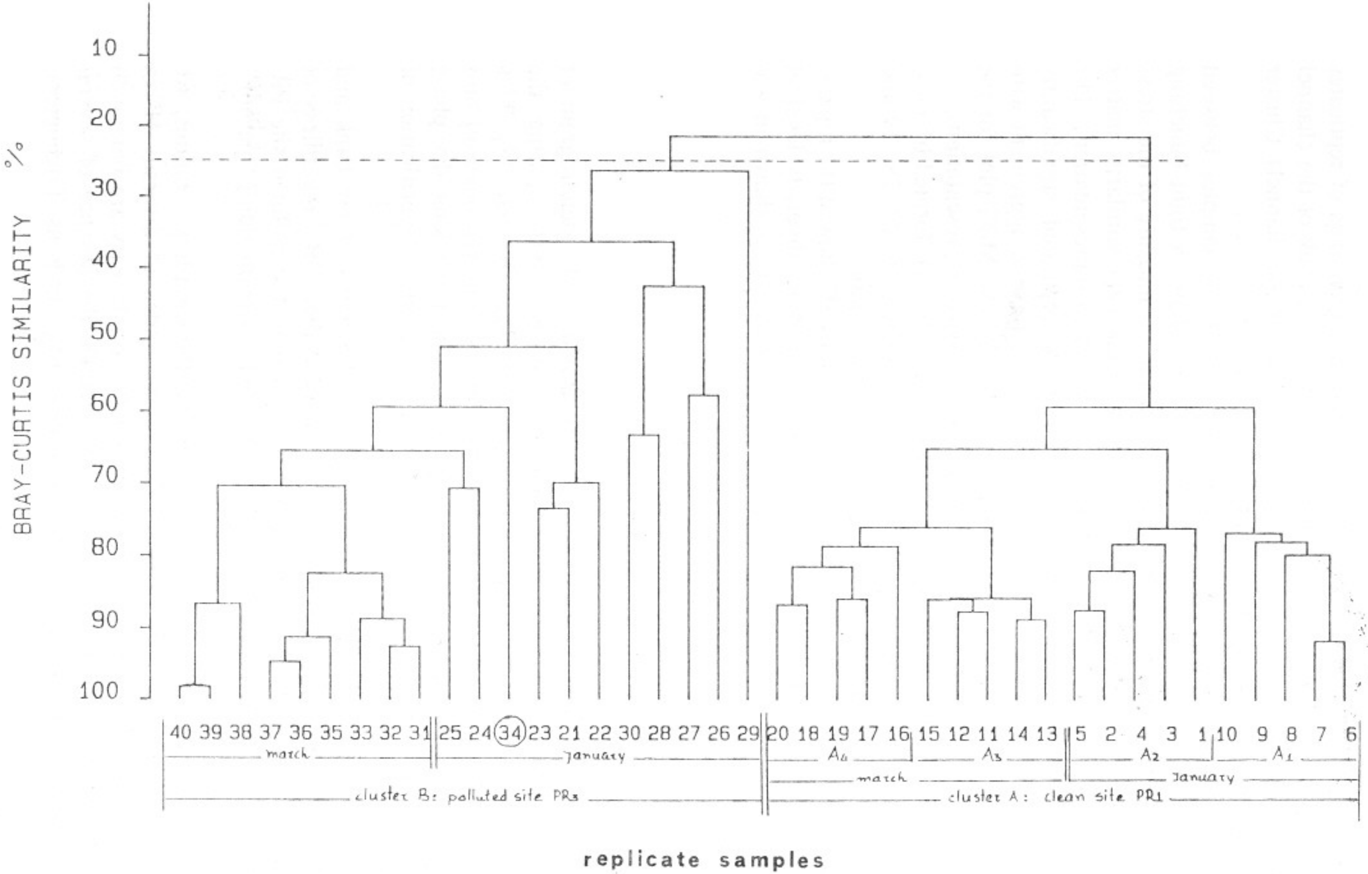

Fig 2. Dendrogram of percentage similarity of faunal composition between 40 replicate samples from two sampling sites for two surveys in 1989, January and March.
The 40 replicate samples, clusters A & B and subclusters A_1, A_2, A_3, A_4 are shown on the horizontal axis (see text).

replicates from the bank of the channel). A_3 and A_4 are groupings of replicates for March (A_3 is a subcluster including replicates from the bank of the channel and A_4 a subcluster including replicates from the middle fo the channel). Cluster B cannot be clearly separated into subclusters.

The multidimensional scaling plot (MDS) (fig. 3) of samples between sampling sites, according to the dendrogram of fig.2 shows a tight bunching of replicates in the control site PR_1. The stress factor - a measure of the stress required to force the two dimensional representation upon the similarity matrix (SPENCE & GRAEF, 1974) - has a value of 0.091 (good representation). The Analysis of Similarity test (ANOSIM) in cluster A, separated significantly ($p<0.05$) the four subclusters, A_1, A_2, A_3, A_4, emphasizing the aggrement with the grouping of replicates in the dendrogram of fig. 2. The MDS plot for the 4 subclusters is presented in fig. 4 (stress 0.071, good representation).

Another assemblage is observed in the MDS plot (fig. 3), formed by most of the replicates of sampling site PR_3 except for replicates 26, 27, 28, 29 and 30 which are isolated on the periphery of the MDS plot.

The value of the Shannon-Wiener index of diversity H' showed the highest diversity of species in control site $PR_1$for March, a medium diversity level in control site PR_1 and site PR_3 for January, while low diversity is shown in site PR_3 for March (fig. 5).

Discussion

The clear separation of clusters A and B shown in the dendrogram of fig. 2, is a sign of a strong environmental "stress" differentiating the macrofaunal populations between the two sampling sites PR_1 and PR_3. This environmental "stress" which is quite possibly coming from the olive oil mill wastes, becomes stronger, due to the low discharge and the long dry phase of the stream, two parameters which do not facilicate the self-purification of the water.

There is also a clear differentiation between the fauna of the bank and that of the middle of the channel in the control station PR_1, regardless of the small channel width. This separation is not clear in the polluted site PR_3 where the high quantities of the wastes disposed into the stream, don't "permit" such a "luxury".

As about the diversity of the species between the sampling stations, we can say that according to fig. 5, the values of the index of diversity H' in the control station PR_1 and the polluted station PR_3 on January are not much different. Looking however in Table 3, we can notice the absence of several groups sensitive to severe pollution from station PR_3, such as Trichoptera,

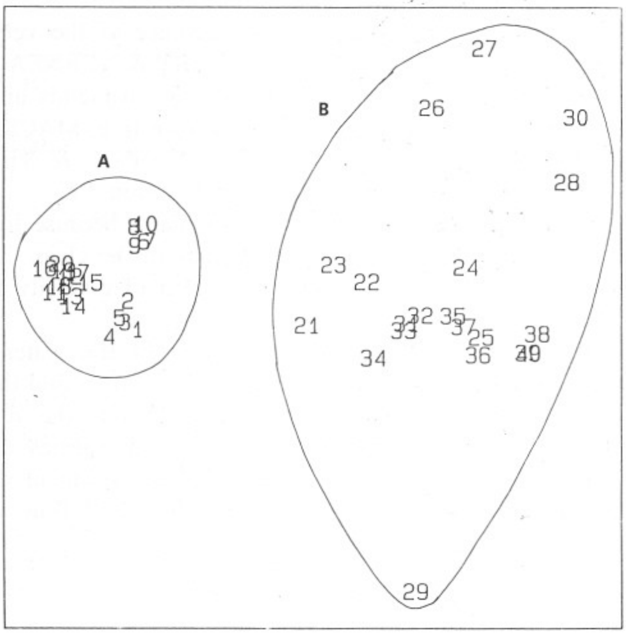

Fig 3. Multidimensional Scaling plot (MDS), showing the separation of cluster A from cluster B, according to the groupings of the dendrogram in fig. 2.

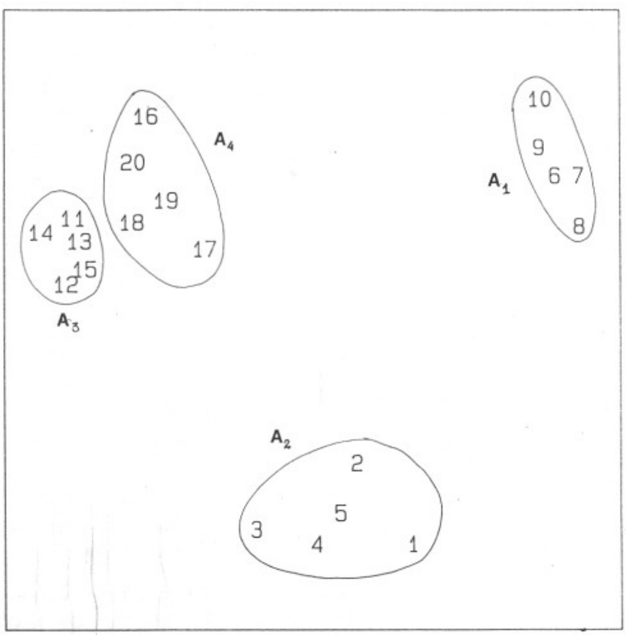

Fig 4. MDS plot of cluster A (station PR_1), of the dendrogram in fig 2, showing subcluster A_1 (Jan, middle), subcluster A_2 (Jan, Bank), subcluster A_3(Mar, bank) and subcluster A_4 (Mar, middle).

Coleoptera and Ephemeroptera, as well as the absence of the very sensitive Plecoptera nymphs (MACKENTHUN, 1969; TUFFERY & VERNEAUX, 1968), groups which are all present in the clean station PR_1. Annelids and dipteran larvae, taxa whose tolerance to pollution is well known (e.g. MACKENTHUN, 1969; WOODIWISS, 1964; CHANDLER, 1970; TUFFERY & VERNEAUX, 1968), are the main taxa present in the polluted station PR_3.

In March, the water in the station PR_3 is stagnant, because in April the stream will be dry. This stagnancy is not so obvious in the clean station PR_1 which is closer to the head of the stream. The index of diversity H' gets its higher value (fig. 5).

.In station PR_3 however, due to the stagnancy of the water, the self-purification of the stream from the previous months' pollution and the recovery of the benthic animal population, are impossible. This is the reason why, although in March the pollution has practically ceased, species diversity in station Pr_3 is very low (fig. 5). The oily covering of the sediment will remain undisturbed and will soon get dry, as in April the water flow will cease.

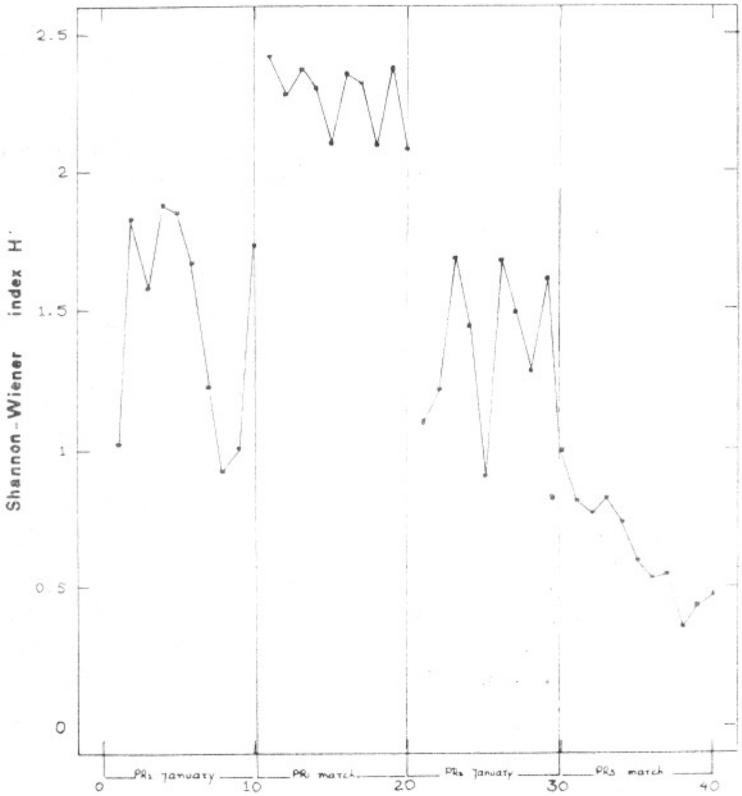

Fig 5. Plot of Shannon-Wiener diversity indices of 40 replicate samples from two sampling stations for two surveys in 1989, January and March (see text). Sampling stations and sampling months are shown on the horizontal axis. Numbers correspond to fig. 2.

Next December, when the waterflow will start again, the recolonization of the sediment by the stream fauna will be difficult. Only the species which are favoured by the pollution will colonize again the polluted part of the stream.

Abstract

In Crete, every year and only during a three month period, 370,000tn of wet wastes frorm olive oil mills are disposed in the environment, most of them reaching intermittent streams.

The deoxygenation of the bottom sediment in parallel with the toxic substances of the wastes (mainly phenols) are the major threat for the macrofauna of running waters.

This paper aims to present the effects of such kind of wastes on the benthic macroinvertebrates of an intermittent shallow stream in Crete.

The reduction of species diversity and abundance and the presence of mainly Oligochaeta and Chironomid larvae, are the signs of a strong ''stress'' on the benthic animal community.

The big load of wastes and the low discharge of the stream in combination with its long dry phase, are the possible factors which prevent the self-purification of the water and the recovery of the benthic invertebrate community.

Περίληψη

Στην Κρήτη κάθε χρόνο και κατά την διάρκεια μιας τρίμηνης περιόδου, 370.000 τόνοι υγρών αποβλήτων αποτίθενται από τα ελαιουργεία στο περιβάλλον και κυρίως στα ρέματα διαλείπουσας ροής.

Η αποξυγόνωση του βενθικού ιζήματος μαζί με τις τοξικές ουσίες των αποβλήτων (κυρίως φαινόλες) είναι οι κύριες απειλές για την μακροπανίδα των τρεχούμενων νερών.

Αυτή η εργασία έχει σαν σκοπό να παρουσιάσει την επίδραση αυτού του είδους των αποβλήτων πάνω στα βενθικά μακροασπόνδυλα ενός ρηχού ρέματος διαλείπουσας ροής της Κρήτης.

Η μείωση της ποικιλότητας των ειδών και της αφθονίας και η παρουσία κυρίως Ολιγοχαίτων και προνυμφών Χειρονομιδών είναι οι ενδείξεις μιας έντονης πίεσης πάνω στην βιοκοινωνία των βενθικών ζώων.

Το μεγάλο φορτίο των αποβλήτων και η χαμηλή παροχή του ρέματος σε συνδυασμό με την μακρά φάση αποξήρανσης, είναι οι πιθανοί παράγοντες

που αποτρέπουν τον αυτοκαθαρισμό του νερού και την αναζωογόνηση της βενθικής ασπόνδυλης βιοκοινωνίας.

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