CA13-005

ISRAEL OCEANOGRAPHIC & LIMNOLOGICAL RESEARCH LTD. חקר ימים ואגמים לישראל בע"מ
THE YIGAL ALLON KINNERET LIMNOLOGICAL LABORATORY P.O.B. 345, TIBERIAS 14102, ISRAEL
המעבדה לחקר הכנרת ע"ש יגאל אלון ת.ד. 345 טבריה 14102, טל' 06-6721444

PN ACH-636
104866

[redacted]

I.O.L.R. REPORTS

SEP 2 1 1999
B

"Migration and transformation of organic chemical compounds in polluted Turkmenistan water bodies"

D. Wynne & R. Parparova (Israel)
V. Viskrebentsev & Y. Fedorov (Turmenistan)
AID/CDR Final Report

Grant No: TA-MOU-94-CA-13-005

T11/99 June, 1999

FINAL REPORT

Covering Period: 25th. January, 1995 to 28th. January, 1999

Submitted to Human Capacity Development
Bureau for Global Programs, Field Support and Research
U.S. Agency for International Development

"Migration and transformation of organic chemical compounds in polluted Turkmenistan water bodies"

Principle Investigators: Dr. D. Wynne & Dr. R. Parparova
Grantee Institution: The Israel Oceanographic and Limnological Research Company Ltd., The Yigal Allon Kinneret Limnological Laboratory, Tiberias, Israel.

Collaborators: Dr. V. P. Viskrebentsev & Eng. Y. Fedorov
Institution: Turkmenistan Ecological Centre, Ashgabat, Turkmenistan.

Project Number: CA-13-005
AID Grant Number: TA-MOU-94-CA-13-005

USAID Grant Project Officer: David Mulenex, Scientific Attache, US Embassy, Tel Aviv, Israel

Project Duration: January 25th.,1995 to January 28th., 1999

Table of Contents

	page
Executive Summary	3
Abbreviations used	4
List of Tables & Figures	4
Research Objectives	5
Introduction	5
Materials & Methods	6
Results and Discussion	8
Conclusions	13
Impact Relevance & Technological transfer	13
Project activities/outputs	14
Project productivity	15
Future work	16
Literature cited	17
Figure Legends	17

Executive Summary

The Gara-Gum canal, situated in Turkmenistan, is 1000 km long and supplies water to a large proportion of the population for domestic and agricultural purposes. Crops grown in the vicinity of the canal include cotton, wheat and grapes. The use of pesticides in these regions is high and such chemical (or their breakdown products) may directly enter the canal. Moreover, several towns and cities along its route may contribute toxic chemicals to the canal waters. The input of xenobiotics into the canal could, therefore, pose a direct and indirect threat to human health. Since there is a high dependency on a clean water supply in many parts of the country, possible pollution of the Gara-Gum canal is viewed with the utmost concern.

During this project, the following studies have been carried out i. The types and quantities of pesticides used in various regions along the Gara-Gum canal have been obtained, ii. analytical techniques for the determination of pesticides in water, soil and sediments have been evaluated for the actual field conditions encountered, iii. seasonal and spatial distributions of organochlorine pesticide residues in the waters of the canal and in the soil in its vicinity have been determined; highest water concentrations were generally found in spring (and summer) although a 60 fold range in levels between stations was observed, iv. concentrations of several organochlorine compounds in the same body of water followed along the entire length of the canal, dropped rapidly but then increased again at other stations, v. pesticide concentrations in the canal water were compared over two consecutive years. Initially, levels of these compounds were high but in subsequent years a sharp decline in concentrations of these compounds was noted at all station. This drop coincided with a reduction in the use of toxic agricultural chemicals in the region around the canal (due to economic pressure), suggesting that there is a strong element of "self-cleaning" in the canal.

In addition to monitoring the canal for levels of pesticide residues, we have also carried out a number of biological experiments. These studies were initially designed to look at the breakdown of pesticides by naturally occurring microorganisms, using the organochlorine insecticides Lindane and Endosulphan as "model" compounds. Unexpectedly, we noted that in several cases, these chemicals could stimulate growth of the organisms; an effect which was both compound and species specific. For bacteria, the presence of the insecticide caused changes in survival, succession and population diversity. These results are important for the proper maintenance of water quality in the Gara-Gum canal, as well as in other water bodies.

Abbreviations used

α, β, γ-HCH: Isomers of hexachlorocyclohexane (benzene hexachloride); [Lindane is the commercial name of the γ isomer.]
DDT: p, p'-dichlorodiphenyltrichloroethane (insecticide)
DDD: p, p'-dichlorodiphenyldichloroethane (degradation product of DDT with insecticidal properties)
DDE: p, p'-dichlorodiphenyldichloroethylene (degradation product of DDT with non-insecticidal properties)
ECD, NPD: Electron Capture and Nitrogen, Phosphorus specific gas chromatograph detectors.
GC: Gas chromatograph; gas chromatography

List of Tables and Figures.

Tables.

1. Seasonal distribution of organochlorine pesticides along the Gara-Gum canal, 1995
2. Soil and water ratios of several pesticides along the Gara-Gum canal.
3. Pesticides, oxygen & organic matter in the Gara-Gum canal, 1995 & 1996.
4. Pesticide concentrations in water and sediment along the Gara-Gum canal, 1997.
5. Pesticides and other chemicals, in a body of water along the Gara-Gum canal, 1995.
6. Chemical parameters, in a body of water along the Gara-Gum canal, 1996

Figures.

1. Map of Turkmenistan, showing the location of the Gara-Gum canal.
2. Photographs of the Gara-gum canal.
3. Soil types, pesticide concentrations and crops grown along the Gara-Gum canal.
4. Temperature and oxygen concentrations along the Gara-Gum canal, 1996.
5. Mineral concentrations along the Gara-Gum canal, 1996.
6. Orthophosphate and inorganic nitrogen concentrations along the Gara-Gum canal, 1996.
7. Iron, silica, BOD_5 and COD concentrations along the Gara-Gum canal, 1996.
8. Pesticide concentrations along the Gara-Gum canal, 1995.
9. Effect of Lindane on the growth of Lake Kinneret phytoplankton.
10. Effect of Lindane and Endosulphan on the growth of Lake Kinneret phytoplankton.
11. Lindane and Endosulphan remaining after incubation with Lake Kinneret phytoplankton.
12. Effect of Lindane on the growth of natural and cultures of Kinneret phytoplankton.
13. Effect of Lindane and sediment on the growth of Kinneret phytoplankton.
14. Effect of Lindane on the growth and succession of Lake Kinneret bacterial populations.
15. Effect of Lindane or an artificial medium on the growth of Kinneret bacterial populations.
16. The effect of Lindane on the growth and survival of *E. coli* species.
17. The effect of Lindane or Endosulphan on the growth and survival of *E. coli* species.

RESEARCH OBJECTIVES

Introduction

Turkmenistan is situated in the southern Central Asian region of the former Soviet Union, adjacent to Iran and Afghanistan (Fig. 1). The country, the capital of which is Ashgabat, is about 500,000 km^2 in area and has a population of approximately 4.5 million people. Turkmenistan became independent in 1991.

Although much of the land in Turkmenistan appears to be desert, some regions have an agricultural potential which has not been utilised solely because of the lack of water. Consequently, the Gara-Gum canal was built to supply water to those areas of possible agricultural significance but where natural sources of water were scarce. Constructed in 1956, the Gara-Gum canal had returned building costs by 1962, mainly from the sale of water for agricultural purposes, although water has also been sold to some domestic users. Only 10% of the Gara-Gum canal water is used for industry.

The Gara-Gum canal, which is approximately 1000 km in length, is considered to be the longest canal built through desert-like regions. The intrusion of salt deposits on the sides of the canal (Fig. 2) also contributes to the pollution of both soil and water but was not within the terms of reference of this project. The canal takes water from the Amudarya river, at Chardzou, and supplies agricultural and domestic water to various cities and settlements en route to Hazanje, close to the Caspian Sea (Fig. 1)[1]. About 60% of the population of Turkmenistan now live in the vicinity of the canal; indeed some villages in the surrounding area have seen a two fold increase in size because of their proximity to the canal. Pollution of this important source of water is, therefore, of the utmost concern.

Cotton, melon and wheat are among the major crops grown in the region around the Gara-Gum canal (Fig. 3) and large amounts of different types of pesticides are used in the area. The application of toxic agricultural chemicals in the immediate vicinity of an important source of water poses a potential direct or indirect danger to human health.

Several factors govern the presence of toxic chemicals in a water body and these in turn, determine the success of the procedure(s) used in their analyses. The compounds might be

---

1 The Amudarya river is not only a major source of water for the Gara-Gum canal but also for the Aral Sea, situated at the end of the river. Flow of water into the canal might have contributed to the drying up of the Aral Sea, but only about 15% of the canal water comes from the river, with the remainder coming from agricultural run-off and other rivers. Scientists in Turkmenistan believe that causes other than the operation of the Gara-Gum canal, have contributed to the "Aral Sea crisis" but further discussion of this problem are beyond the scope of this project.

highly water-soluble (and, therefore, not easily extractable with an organic solvent); they may breakdown completely in the aquatic environment (or it may not be possible to detect their breakdown products by conventional techniques). This project was, therefore, carried out to evaluate the potential threat of agricultural compounds on the chemistry and biology of the Gara-Gum canal ecosystem in more detail and to propose recommendations to the Turkmenistan government to reduce, or eliminate, pollution of such chemicals in the area.

Materials and Methods

Samples (1 liter of water; 10g $_{(dry\ weight)}$ of soil or sediment) were collected at regular intervals from 11 - 17 stations along the Gara-Gum canal. Water samples were taken with a water sampler from 0.2m depth; soil and sediment with a corer or dredge sampler).

Because of its length, two approaches were used to sample the Gara Gum canal: i. At several times during the year, about 15 stations situated along the length of the canal were sampled simultaneously, or ii. The same body of water was followed from the beginning to the end of the canal, which was sampled every 100km or so (corresponding to the stations in i, above.)

For pesticide analyses, all samples were extracted with an organic solvent (usually hexane) and the extract reduced in volume on a rotary evaporator. The extracts were cleaned up on a column of Florisil, if necessary and pesticide residues analysed by gas chromatography (GC). Polychlorinated biphenyl compounds (PCBs), which interfere with the analysis of organochlorinated pesticides, were dechlorinated with potassium hydroxide before injection into the GC.

In Turkmenistan, GC analyses were carried out at the Centre for Ecological Monitoring, Ashgabat, with a Sviet gas chromatograph, fitted with an electron capture detector (ECD). Separations were carried out on packed 1-2m glass columns (0.2-0.4cm i. d.) using various column packings (such as 5% SE30 or XE60 on Chromatone N-AW-DCMS; mixtures of QF1, OV1 (or OV11 & OV17) and XE60 on Chromosorb W-HP). Standards of α-, β- and γ-HCH; Heptachlor, Heptachlor epoxide; Aldrin; Dieldrin; DDD; DDE; DDT and PCBs (Arochlors 1221, 1242, 1254, 1260 or their analogues) were used to identify and quantitate the compounds detected.

At the Kinneret Limnological Laboratory, GC analyses were carried out using a Perkin-Elmer Turbochrome gas chromatograph, also fitted with an ECD and a nitrogen-phosphorus

specific detector (NPD). Glass capillary columns (30m; 0.32mm i. d.) with specific liquid phases for the separation of pesticide residues (SE54 or AT1701; both obtained from Alltech, Inc.) were used in this study.

Several laboratory experiments were carried out at the Kinneret Limnological Laboratory to study the interactions between pesticides and the environment. Such experiments included both the positive and negative influences of pesticides on the biota and a study on biotic and abiotic breakdown of selected "model" pesticide compounds.

The effect of the organochlorine insecticides Lindane and Endosulphan (at 5 to 10μg l^{-1}) on algal growth was carried out, as follows. Natural phytoplankton populations from the lake were first prescreened on 300μm netting to remove zooplankton and large detrital particles and the water supplemented with inorganic N and P (in a 10:1 molar ratio) as nitrate and orthophosphate, respectively, to maintain growth. The samples were then exposed to the insecticides (either separately or together) and growth followed. The same experiment was also carried out with monoalgal cultures from the Kinneret Limnological Laboratory culture collection. Cultures of *Peridinium gatunense* and *Pediastrum* sp., maintained on Lindstrom 16 (Lindstrom, 1991) or SCM (Moss, 1972) media, respectively, were transferred to filtered (0.45μm) Kinneret water, supplemented with nutrients as above. In some experiments, sediment (1g l^{-1}) was added to the system inside a dialysis tube and the pesticide injected directly into the sediment.

In all experiments, growth was followed by measuring Chlorophyll a concentrations with a fluorimetric method, after extraction into 90% acetone (Holm-Hansen et al., 1965). All experiments were carried out at 18°C, under continuous light (50-100μEin s^{-1} m^{-2}). At the end of the experiments the samples were extracted with hexane and the concentration of insecticide remaining was determined by gas chromatography.

The effect of these insecticides was also carried out on both natural populations and pure cultures of bacteria. Lake water was taken from a pelagic station at various times of the year and incubated at 37°C in the presence and absence of the pesticides. At daily intervals a sample was taken and direct counts determined by counting DAPI stained cells under an epifluorescent microscope (Porter & Feig, 1980). An aliquot was also plated onto agar dishes (PCA; APHA, 1992) and colony forming units (cfu) counted after 24h incubation at 37°C. In some cases, the colonies were differentiated from each other on the basis of their size and/or colour.

The effect of pesticides on bacterial growth was also carried out with cultures of bacteria, isolated from Lake Kinneret. Two strains of *E. coli* - the "wild type" (#1) and one containing a plasmid (an extra-nuclear piece of DNA), possibly conferring resistance to toxic substances (#47), were grown in LB medium (Atlas, 1993), in the presence and absence of pesticides. Growth of the bacteria were followed by plating on LB medium and the culturable cell numbers (cfu) counted after incubation of the plates at 37°C for 24 hours (APHA, 1992).

Results and discussion.

Seasonal and spatial distribution of organochlorine pesticides in the Gara-Gum canal.

In the first year of our studies (1995) both seasonal and geographical differences in pesticide concentrations were observed (Table 1). Highest pesticide concentrations were generally found during spring (and sometimes stretching into summer) although a large range in concentrations was noted between stations. For example, concentrations of both α- and γ-HCB at Zachmet, Mary and Dushak were about 0.01μg l^{-1}; whereas at Kodj, water concentrations of these compounds reached 0.6 and 0.2μg l^{-1}, respectively.

These results probably reflect the types of pesticides used and the crops grown at the sites sampled. The type of soil along the Gara-Gum canal changes dramatically from one end to the other and this is reflected in the different crops grown in the region. The pesticides in use in the canal region may, therefore, vary considerably from place to place. These differences were also noted in the concentrations of pesticides in the soil (Fig 3a-d). For certain compounds, however, the soil : water ratios were constant, even though there were changes in the absolute concentrations found at these sites (Table 2). Furthermore, although DDT (and DDE, to a lesser extent) levels in soil were quite high, the concentration of these compounds in water samples from the same sites, were below the limit of detection. These results suggest that at least some of the toxic chemicals found in soil will not be released into the surrounding aquatic environment when the soil particles reach the canal waters.

During 1996, only trace levels of pesticides were detected in the samples, except those from Kodje and have been compared to those found in 1995 (Table 3). In general, α, β and γ-BHC were usually found, but sometimes DDT was also detected. For 1997, pesticide residues were not detected in samples from every site (Table 4). In positive samples, the insecticide Lindane (γ-HCH) was found in both water (2-60μg l^{-1}) and sediment (0.3- 4μg kg^{-1}). DDE (a breakdown product of DDT) was also detected in a few sediment samples (~4μg kg^{-1}). It

should be noted that the application of various pesticides was much higher in 1995 than in subsequent years, due to economic considerations, and in some areas the local farmers used no pesticides at all. This explanation accounts for the low levels of pesticide residues found in water samples during 1996 and 1997. Our data suggests, however, that the canal waters are not able to accumulate pesticide residues, since their concentrations dropped considerably almost immediately after pesticide use had been discontinued.

Regional trends in general water parameters along the Gara-Gum canal.

As well as pesticide residues, the canal waters were analysed for a further 26 chemical parameters, including those used as indicators of water quality and the "well being" of water bodies (such as O_2, BOD_5, organic matter and inorganic nutrient concentrations). Values for some of these parameters were relatively constant along the entire length of the canal, showing little annual and inter-annual variability. However, although oxygen concentrations and biological oxygen demand (BOD), which measures the availability of organic matter to micro-organisms, in the canal were similar for both 1995 & 1996, a large increase in chemical oxygen demand (COD) was noted in 1996, for all stations (Table 3). An increase in COD levels, at constant BOD values, suggests that the rise in organic matter concentration in the canal is mostly biologically unavailable. Some of the sharp fluctuations in COD may also indicate that sewage was entering the canal at various sites.

Distinct regional trends in the annual averages of some of the chemical and physical parameters were noted and representative data (for 1996) are given (Fig. 4-7). Some of the parameters, such as temperature, oxygen concentrations and % O_2 saturation, did not vary significantly along the canal and averaged 26.1±1.5°C, 9.49±0.76 mg l^{-1} and 118±9%, respectively (Fig. 4). Other parameters showed increased levels at some stations and decreased levels at others. For example, although absolute concentrations of various inorganic minerals were different from each other, their pattern along the canal was similar (Fig. 5). These ions tended to increase at the Tedjen Dam, Dushak and Gazanjik stations but were low at other sites. Such a pattern was much more noticeable in the inorganic nitrogen concentrations (Fig. 6) and in the silicon & COD levels (Fig. 7), where sharp fluctuations in these levels could be discerned. It should be noted that levels of SRP & nitrite (Fig. 6) and iron & BOD (Fig. 7), were generally low along the entire length of the canal and temporal patterns in those nutrients were more difficult to determine.

Chemical constituents in a water body flowing along the Gara-Gum canal

In view of the logistics involved in sampling a canal nearly 1000km long, we used a second approach to obtain samples for this project. Samples were taken at the regular sampling site (Halounoe) where the Amudarya river enters the canal, then the same body of water was followed to the end of the canal at Gazanjik. Water was sampled approximately every 100km, as well as from 3 reservoirs, and analysed for organochlorine pesticides as well as a number of other parameters, such as oxygen content, nutrients, BOD & COD, temperature and water velocity. The results are summarised in Table 5. Little change was observed in both water temperature and flow rate along the canal (averaging 22.9°C and 0.5 m s^{-1}, respectively during 1995), although water flow tended to be higher at the beginning of the canal. Temperatures were significantly higher in 1996 in comparison to 1995. In samples from several stations, pesticide concentrations were high during 1995. For example, elevated α- and γ-HCH levels were found at the beginning of the canal, then decreased. Surprisingly, high pesticide concentrations were found near the Kaaka and Ashgabat stations, which essentially represent urban areas (Fig. 8). DDE (a breakdown product of DDT) levels peaked at the Ashgabat and Kopetdag stations (Fig. 8).

Effect of selected pesticides on the growth of micro-organisms.

Several laboratory experiments were designed to examine the breakdown of pesticides under natural environmental conditions in the canal. Unfortunately, it was not possible to carry out such experiments in Turkmenistan due to technical and logistic reasons. Instead, studies were carried out at the Kinneret Limnological Laboratory, using "model" organochlorine insecticides in use in both Israel and Turkmenistan. We noted that instead of the anticipated toxic effects of these compounds, they often, unexpectedly, had a positive effect on the growth of micro-organisms (algae and bacteria) and some preliminary results of this aspect of environmental pesticide pollution are presented in this report.

Phytoplankton

Water samples were taken from the Kinneret in October 1996; October 1997 and February 1998 and incubated with and without Lindane. In 1996 & 1997, growth of natural phytoplankton populations (as followed by chlorophyll concentrations) was greater in the

presence of the insecticide (Fig. 9). In February 1998 there was an initial growth phase then the algae appeared to die off. Again, the cells grew better in the presence of Lindane (Fig. 9).

We also compared the effect of two organochlorine insecticides (Lindane and Endosulphan) on the growth of natural phytoplankton populations. Lake water was incubated for several weeks with Lindane (10μg l^{-1}), Endosulphan (10μg l^{-1}) or a mixture of both (5μg l^{-1} each) and growth followed. Initially, algal growth was not influenced by the presence of Endosulphan whereas Lindane was a strong inhibitor (Fig. 10). However, gas chromatograms showed that Lindane concentrations in the water were still high at the end of the experiment, whereas those of Endosulphan had dropped to undetectable levels (Fig. 11). The disappearance of Endosulphan from the water phase could explain its differential effect on algal growth, in comparison to Lindane. It is possible that the input of Endosulphan into a water body (such as the Kinneret or the Gara Gum canal) may not effect algal growth, although the effects of the insecticide on the entire ecosystem also need to be taken into consideration.

The influence of Lindane on natural phytoplankton populations was also compared to its effect on algal cultures. As described above, the algae were grown under controlled conditions in the laboratory, then transferred to filtered Kinneret water for the experiment. We chose *Peridinium gatunense*, the bloom forming dinoflagellate, and *Pediastrum duplex*, a green alga, as "representative" of the phytoplankton from the Kinneret. Moreover, since the usual bloom of *Peridinium* in the Kinneret did not occur during 1997, we had to use laboratory cultures for this aspect of the work. As has been reported above, natural phytoplankton populations grew better in the presence of the insecticide (Fig. 12A). After a typical lag period, however, both the growth and cell yield of *Peridinium* were much lower in the presence of Lindane (Fig. 12B). *Pediastrum* also showed a long lag period but again Lindane exerted a toxic response and completely inhibited algal growth (Fig. 12C).

Since Lindane is known to bind strongly to sediment particles, we carried out the same experiment in the presence and absence of sediment. Even though there was a long lag period, the phytoplankton grew well in the presence of sediment (Fig. 13), possibly due to the supply of biologically available sedimentary phosphorus (Wynne et al., unpublished results). In contrast to previous findings, Lindane inhibited phytoplankton growth in this experiment, although its toxic effect was not dependant on the presence of sediment. From gas chromatographic determinations, we noted that the amount of Lindane in the water phase was also unaffected by sediment. The factors affecting pesticide binding capacity of sediments

(such as type and quantity of the inorganic and organic components, granulometry of the particles, temporal & seasonal changes in these parameters etc.) are complex and are not fully understood, but clearly influence the toxicity of xenobiotic compounds in a water body.

Bacteria

The influence of organochlorine insecticides on natural bacterial populations from the Kinneret was also examined and some representative results are presented here. Bacterial numbers were evaluated in two ways. Total bacterial numbers were determined by counting DAPI stained cells under the epifluorescence microscope. This is a rapid and simple procedure. We also counted cell numbers as colony forming units (cfu) after plating on selective growth media. Even though this method ignores non-culturable bacterial forms and is time consuming to set up and obtain results, it enabled us to observe changes in succession in bacterial populations, possibly induced by the presence of the insecticide. Consequently, this was the method chosen to determine bacterial numbers in our experiments. Since these bacterial species have not yet been completely identified, however, results have been presented on the basis of colony size and/or colour on the agar plates.

The growth and succession of natural bacterial communities was influenced by the presence of Lindane (Fig. 14). Some sub-populations of bacteria were unaffected by the insecticide ("White"; Fig. 14A), whereas others grew better in the presence of Lindane (e.g. "Brown"; Fig. 14B). The succession of some species (e.g. "Large white" and "Large Yellow"; Fig. 14-D & E) was changed in the presence of Lindane.

We compared the growth of natural bacteria populations on Lindane and on an artificial growth medium (LB; Lactose Broth) as sole carbon sources. During April, the cells grew on Lindane just as well as they did on LB (Fig. 15-A) and this pattern was even more marked in the experiment conducted during May (Fig. 15-B). These results suggest that natural bacterial populations possess the appropriate enzymatic mechanism(s) to breakdown Lindane and to use it as a convenient carbon source.

The effect of insecticides on a pure culture of *E. coli,* isolated from the Kinneret, was also investigated. Two strains of the bacterium were used. One (designated n1) contained an extra-nuclear piece of DNA (a plasmid) which is thought to confer resistance to antibiotics to the cells. The second species (designated n47), did not possess the plasmid. The bacteria were grown in an artificial medium, then inoculated into filtered (0.2μm) Kinneret water in the presence and absence of Lindane or Endosulphan. The plasmid containing bacterium

grew better in the presence of Lindane than the bacterium lacking the plasmid (Fig. 16). We noted differences between sampling dates (Fig. 16), suggesting that the processes involved in the utilisation of Lindane are also dependent on environmental factors. A similar pattern was also noted for Endosulphan. In this case, the survival of the plasmid containing bacterium (n1) was much higher in the presence of the insecticide (Fig. 17).

Conclusions

During the first year of this project we detected pesticide residues in most of our samples and at nearly all of the sampling stations. Particularly worrying was the input of several pesticides into the canal from urban stations, presumably via sewage contamination.

The use of agricultural chemicals in Turkmenistan then declined in the later years of the project, primarily due to economic considerations, and was reflected in the types and levels of pesticides detected in the water and sediment samples from the canal during this phase. When the external input of pesticides was reduced or halted, only Lindane and DDE (both of which are considered very environmentally persistent) could be detected, suggesting that the canal ecosystem may be able to quickly clean itself from pesticide residues. However, we have also noted that at some sites the levels of organochlorine pesticides in the soil and sediment was quite high, even when water concentrations were low. These particulate fractions represent a hidden potential danger to agriculture or human health since the chemical may be released to the water or biota under the appropriate conditions or might still be toxic in the bound form. Indeed, preliminary experiments revealed that Lindane exerts a toxic response to phytoplankton even when mixed with Kinneret sediments. Further work is required to examine this point more closely and to determine the possible factors involved in binding and release of pesticides to particles.

From our experiments with micro-organisms, we have noted that low concentrations of insecticides might have either a positive or negative effect on growth and/or succession of both natural populations or cultures of algae and bacteria. The presence of such chemicals in a water body should, therefore, be viewed with the greatest concern, especially if it is a source of water for domestic or agricultural purposes (such as water from the Kinneret or the Gara-Gum canal). Moreover, the possible involvement of plasmids (thought to confer antibiotic resistance to the cells) in bacterial pesticide metabolism may be a cause for alarm and further experiments are necessary to explore this aspect of our findings.

Impact relevance and technology transfer

The findings for this project will be useful to Turkmenistan in a number of ways. Firstly, we have determined the seasonal changes of pesticide concentrations in the waters of the Gara-Gum canal and followed these waters as they flow along the 1000km length of the canal. This has enabled us to come to a number of operational conclusions - important because of the use of Gara-Gum waters for agriculture and domestic purposes. Since this project is based on long term field and experimental work, the interim results have not been used so far. It is anticipated, however, that this report (and previous ones) will form the basis of recommendations to the Turkmenistan government to prevent future deterioration in the water quality of the Gara-Gum canal.

Since the Kinneret Limnological Laboratory is carrying out a long-term pesticide monitoring program of the Lake and its drainage basin, the technology used in this work is being continuously refined. For example, water extraction methods have been improved and the use of other techniques (such as solid phase extraction). The CEM also benefited from these improvements as part of the technological transfer required in this project.

Project activities/outputs

Lectures: Part of the results from this project have been presented at the following meetings:

i. A lecture was presented at "The International Water Resource Conference", Herzlia, Israel, November, 1996.

ii. A lecture was presented at the "International Society for Pure & Applied Limnology (SIL)" meeting, Dublin, Ireland, August, 1998.

iii. A poster was presented at the "Kinneret Symposium", Kibbutz Ginosar, Israel, September, 1998.

iv. Two lectures were presented at the "SECOTOX" (International Society for Toxicology) meeting held in Antalya, Turkey, October, 1998.

v. A lecture was presented at the "Israel Ecological Congress", Jerusalem, Israel, June, 1999.

Publications: Two papers (in Russian), based on the results of this study, are currently in press in the journal "Pastenyi Turkmenistan" (Turkmenistan Desert).

The paper presented at the SIL meeting in Dublin has been accepted for publication in the

proceedings of the meeting (after undergoing review).

Papers currently submitted for publication:
One lecture given at the SECOTOX meeting has been submitted to the journal "Science of the total environment" and the other has been submitted to "Water Science & Technology". Both are currently undergoing review.

Visits: The Turkmenistani PI's made several visits to Israel during the first two years of the project. During these visits, the scientists studied current methodology in use in the Kinneret Limnological Laboratory for the analysis of environmentally persistent pesticide residues. Due to differences in analytical capabilities between the two laboratories, however, these methods had to be adapted to suit the equipment currently available in Turkmenistan. During the initial phases of the project, the Israeli PI's traveled to Turkmenistan to visit the Center for Ecological Monitoring laboratory in Ashgabat. During one of these visits the Israeli scientists visited the Gara-Gum canal and the Kopet-Dag reservoir, in the vicinity of Ashgabat. At the same time, data and reports were discussed and possible technical problems in carrying out future analyses were evaluated. Dried sediment samples from part of the canal and from several reservoirs were collected and taken back to Israel for comparative analysis, using the standard procedures for such materials employed at the Kinneret Limnological Laboratory.

Project productivity: The project accomplished most of the goals set out in the original proposal. However, several aspects of the project could not be completely carried out due to technical or logistic reasons.

a. The use of pesticides in the entire Gara-Gum region declined drastically during the course of this investigation because of economic pressures on the farmers. Such a situation was unforeseen at the beginning of the project but enabled us to discover that within a short period of time, levels of organochlorine pesticides in the canal waters and surrounding fields dropped to below the levels of detection. Because of this, however, some of the implications we made during the first two years of the study, could not be corroborated during the remaining period.
b. The use of persistent pesticides in Turkmenistan (as in the rest of the world) is declining

in favour of more "environmentally friendly" chemicals (such as organophosphorus and carbamate compounds). For the analyses of such compounds, however, a GC with an N, P specific detector (NPD) and/or an HPLC is necessary, which was unavailable at the Center for Ecological Monitoring. Analytical equipment was only available to measure levels of organochlorine pesticide residues. [It should be noted, though, that generally organophosphorus and carbamate compounds breakdown rapidly in the aquatic environment, which would exacerbate the logistic problems of sampling a canal nearly 1000Km in length.]. Moreover, levels of other toxic agricultural chemicals (such as herbicides) in the Gara-Gum canal waters were also not measured in this study.

c. Technical difficulties made it necessary to carry out the biological experiments in the Kinneret Limnological Laboratory. The interactions between pesticides and micro-organisms were, therefore, carried out using "model" compounds that are in use in both Turkmenistan and Israel.

d. The transformation and/or breakdown of pesticide residues found in the Gara-Gum canal is still not fully understood. This complex aspect of these studies involves the interaction of many chemical, physical and biological factors that have not yet been completely resolved.

Future work: As in all scientific studies, it is anticipated that several aspects of our study will lead to additional projects. a. Studies on the concentrations of toxic agricultural chemicals other than organochlorine insecticides, and an assessment of their environmental impact, in the Gara-Gum canal are required. b. Hydrodynamic and other physical studies on the input of particles and particle-bound chemicals should be investigated. c. The biotic and/or abiotic transformation of toxic agricultural chemicals under the actual conditions found in the region also requires further study. d. The socio- economic aspect of pesticide use, especially in developing countries, requires further study. Since most of our knowledge on this topic is based on experience in developed countries, with a sound economic structure, the relevance to farmers in Turkmenistan is low. [AID, and other international funding agencies, could provide the impetus for more global research on this topic.]

Acknowledgements

We thank Dr. T. Bergstein Ben-Dan, The Yigal Allon Kinneret Limnological Laboratory, for assistance with the bacteriological experiments described in this report.

Literature cited

APHA. (1992). Standards methods for examination of water and wastewater. 18th ed. Greenberg, A. E., Clasceri, L. S. & Eaton, A. D. [eds.], American Public Health Association. pp. 932-934.

Atlas, R. M. 1993. Handbook of Microbiological Media [Ed: I. C. Parks] CRC Press, Boca Raton, p523.

Holm-Hansen, O., C. Lorenzen, R. W. Holmes & J. D. H. Strickland 1965 The fluorimetric determination of Chlorophyll. J. Cons. Cons. Int. Exp. Mer. 30: 3-15.

Lindstrom, K. 1991 Nutrient requirements of the dinoflagellate *Peridinium cinctum*. J. Phycol. 27: 207-219.

Moss, B. 1972. The influence of environmental factors on the distribution of freshwater algae: an experimental study. J. Ecol. 60: 917-932.

Porter, K.G. & Feig, Y.S. 1980 The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25: 943-948.

Wynne, D., Z. Hu & T. Bergstein Ben-Dan. The Influence Of Artificially Induced Salinity Changes On Lake Kinneret (Israel) Micro-Organisms. Verh. Internat. Verein. Limnol. 27 (in press).

Wynne, D., R. Parparova & T. Bergstein Ben-Dan. The influence of Organochlorinated Insecticides on the Growth of Micro-organisms from Lake Kinneret, Israel. (in press)

Figure Legends

Fig. 1. Map of Turkmenistan, showing the location of the Gara-Gum canal.

Fig. 2. The Gara-gum canal, in the region of Ashgabat and the Kopetdag reservoir. (Note the heavy salt deposits in the canal walls) *[Photographed by Dr. David Wynne, The Yigal Allon Kinneret Limnological Laboratory]*

Fig. 3a-d. Soil types, pesticide concentrations ($\mu g\ kg^{-1}$) and crops grown along the Gara-Gum canal. [Pesticide residues are 1 = α-HCB; 2 = γ-HCB; 3 = β-HCB; 4 = DDE; 5 = DDT]. (Data for 1995)

Fig. 4. Temperature, oxygen concentrations and % oxygen saturation at various sites along the Gara-Gum canal, 1996.

Fig. 5. Mineral concentrations at various sites along the Gara-Gum canal, 1996.

Fig. 6. Orthophosphate and inorganic nitrogen concentrations at various sites along the Gara-Gum canal, 1996.

Fig. 7. Iron, silica, BOD_5 and COD concentrations at various sites along the Gara-Gum canal, 1996.

Fig. 8. Changes in water concentrations ($\mu g\ l^{-1}$) of DDE, α- & γ-HCB in a single water body followed along the Gara-Gum canal.

Fig. 9. Growth of natural phytoplankton populations from Lake Kinneret in the absence (□) or presence (■) of Lindane. Initial sampling dates were 9.10.96 (A), 6.10.97 (B) and 20.2.98 (C).

Fig. 10. The effect of Lindane and Endosulphan on the growth of natural phytoplankton populations from Lake Kinneret.

Fig. 11. Gas chromatograms of Lindane (a & c) and Endosulphan (b & d), before (a & b) and after (c & d) incubation with Lake Kinneret phytoplankton.

Fig. 12. Growth of phytoplankton from Lake Kinneret in the absence (□) or presence (■) of Lindane. A: mixed population from the lake (October); B: *Peridinium gatunense* and C: *Pediastrum duplex*.

Fig. 13. Influence of sediment ($0.5g\ l^{-1}$) on the growth of Kinneret phytoplankton in the absence (□) and presence (■) of Lindane.

Fig. 14. Growth and succession of natural bacterial populations from Lake Kinneret in the absence (□) or presence (■) of Lindane. The bacteria were differentiated according to their size and/or colour (see text for more details). A: "White"; B: "Red"; C: "Brown"; D: "Large White"; E: "Large Yellow".

Fig. 15. Growth of natural bacterial populations in the absence (□) and presence of an artificial medium (LB; ▨) or Lindane (▩). A: April; B: May.

Fig. 16. The effect of Lindane on growth and survival of *E. coli* species, lacking (n47) or containing (n1), a plasmid. [See text for more details.].

Fig. 17. The effect of Lindane or Endosulphan on growth and survival of *E. coli* species, lacking or containing a plasmid. [See Fig. VII, and the text, for more details.].

Table 1. Average seasonal distribution of organochlorine pesticides (μg l^{-1}) in stations from the Gara-Gum canal, 1995

	Season			
Station/pesticide	winter	spring	summer	Autumn
Holovnoe				
α-HCB	0.04	0.159	0.101	0.047
γ-HCB	0.02	0.126	0.065	0.027
Heptachlor	0.06	0.045	0.012	-
Zachmet				
α-HCB	0.006	0.009	0.005	-
γ-HCB	0.005	0.007	-	-
Heptachlor	-	0.015	-	-
Mary				
α-HCB	0.007	0.005	0.025	-
γ-HCB	0.004	0.005	0.016	-
β-HCB	-	-	0.009	-
Heptochlor	0.012	0.020	0.039	-
Dushak				
α-HCB	0.005	0.007	0.010	-
γ-HCB	0.005	0.007	-	-
Heptachlor	-	0.014	-	-
Kaaka				
α-HCB	0.005	0.008	0.010	-
γ-HCB	0.004	0.006	-	-
Heptachlor	-	0.021	-	-
Ashgabat				
α-HCB	0.005	0.009	0.035	-
γ-HCB	0.003	0.013	-	-
DDE	-	-	0.005	-
Kopetdag				
α-HCB	0.007	0.003	0.050	0.007
γ-HCB	0.003	-	0.015	0.005
β-HCB	0.004	-	0.011	0.018
DDD	-	-	-	0.193
Heptachlor	-	0.087	0.021	-

Table 1 (cont.)

Kodj				
α-HCB	0.023	0.166	0.183	0.014
γ-HCB	0.014	0.124	0.092	-
β-HCB	-	-	0.017	-
DDE	-	-	0.111	-
Gazangik				
α-HCB	0.044	0.173	0.098	0.011
γ-HCB	0.016	0.085	0060	-
β-HCB	-	-	0.010	-
DDE	-	-	0.051	-
Hanhovus reservoir				
α-HCB	-	0.011	0.145	-
γ-HCB	0.007	0.013	0.020	-
β-HCB	-	0.008	-	-
Heptachlor	-	0.016	-	-
Kopetdag reservoir				
α-HCB	0.005	-	-	-
γ-HCB	-	-	0.009	0.004

-: not detected

Table 2. Ratio between soil and water concentrations of several pesticides, at various sites along the Gara-Gum canal*.

Station	Pesticide			
	α-HCB	γ-HCB	DDE	DDT
Halovnoe	0.0012; 0.006 0.20	0.0006; 0.017 0.035		
Mary	0.0016; 0.008 0.20	0.0009; 0 -	0.0029; 0 -	
Dushak	0.0013; 0.007 0.19	0.001; 0 -	0.0048; 0 -	0.0084; 0 -
Ashgabat	0.0019; 0.007 0.27	0.0015; 0.023 0.065	0.0084; 0.07 0.49	0.0076; 0 -
Hazularbat	0.0004; 0.014 0.28	0.0005; 0.009 0.06	0.0043; 0.001 4.3	

*Values are given for the concentration of various pesticides in soil (mg kg^{-1}) and in water (μg l^{-1}) with the soil/water ratio ($x10^{-3}$) under each pair, where applicable.

Table 3. Concentrations of pesticides, oxygen & organic matter in water from various sites in the Gara-Gum canal, 1995 & 1996.

Station	Year	Pesticide concentrations ($\mu g\ l^{-1}$)						Oxygen ($mg\ l^{-1}$)			
		α-BHC	γ-BHC	β-BHC	DDT	DDD	DDE	O_2	BOD_5	COD	COD/BOD
Halounoe	1995	0.006	0.017	0	0	0	0	7.85	0.93	17.3	18.6
	1996	Traces	traces	traces	n.d.	n.d.	n.d.	8.74	0.93	37.6	40.4
Zahmet	1995	0.009	0.007	0	0	0.006	0	7.45	1.09	9.0	8.3
	1996	traces	traces	traces	n.d.	n.d.	n.d.	9.01	0.83	56.4	68.0
Mary	1995	0.008	0	0	0	0	0	7.50	0.83	13.5	16.3
	1996	traces	traces	traces	traces	n.d.	n.d.	9.58	1.12	30.1	26.9
Dushak	1995	0.007	0	0	0	0.001	0	7.60	1.01	15.4	15.2
	1996	traces	traces	traces	n.d.	n.d.	n.d.	8.92	0.92	30.1	32.7
Kaaka	1995	0.011	0.017	0	0	0	0.002	7.63	0.73	19.2	26.3
	1996	0.014	traces	traces	n.d.	n.d.	n.d.	8.23	1.10	52.6	47.8
Ashgabat	1995	0.007	0.023	0	0	0	0.007	8.15	1.17	13.1	11.2
	1996	traces	traces	traces	0.007	n.d.	n.d.	9.22	1.03	37.6	36.5
Kopetdag	1995	0.002	0.004	0.018	0	0	0.007	8.10	1.19	18.9	15.9
	1996	traces	traces	traces	n.d.	n.d.	n.d.	10.05	1.03	56.4	54.8
Kodj	1995	0.042	0.026	0.018	0	0	0.005	8.20	1.06	14.9	14.1
	1996	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10.20	0.82	45.1	55.0
Gazanjik	1995	0.014	0.009	0.007	0	0	0.001	8.08	1.32	15.9	12.0
	1996	traces	traces	traces	n.d.	n.d.	n.d.	11.33	1.14	45.1	39.6

Table 4. Pesticide concentrations in water and sediment along the Gara-Gum canal, 1997.

	Pesticide concentrations		
	Water ($\mu g\ l^{-1}$)	Sediment ($\mu g\ kg^{-1}$)	
Station	γ-HCB	γ-HCB	DDE
Golovnoe	-	-	-
Mary	-	-	-
Hanhovus	0.0006	0.0009	-
Tedjen Br.	0.005	0.0005	-
Tedjen	0.009	0.006	-
Kaahka	-	0.0005	-
Ashgabat	0.009	0.0006	0.0039
Pumping station	0.0039	0.0009	0.0038
"837"	0.058	0.003	-
"851"	0.009	0.0016	0.0030
"969"	-	-	-

Table 5. Changes in pesticides and other parameters, in a body of water flowing along the Gara-Gum canal.

Station	Date & time	T (°C)	Pesticide concentrations ($\mu g\ l^{-1}$) α-HCB	γ-HCB	β-HCB	DDT	DDD	DDE	O_2	BOD_5 ($mg\ l^{-1}$)	COD	COD: BOD
Halounoe	24.08 10:00	22.2	0.006	0.017	0	0	0	0	7.85	0.93	17.3	18.6
Zahmet	31.08 06:00	23.4	0.009	0.007	0	0	0.006	0	7.45	1.09	9	8.3
Mary	02.09 22:00	25	0.008	0	0	0	0	0	7.5	0.83	13.5	16.3
Dushak	07.09 05:00	23.2	0.007	0	0	0	0.001	0	7.6	1.01	15.4	15.2
Kaaka	08.09 04:00	23.1	0.011	0.017	0	0	0	0.002	7.63	0.73	19.2	26.3
Ashgabat	11.09 09:00	22.9	0.007	0.023	0	0	0	0.007	8.15	1.17	13.1	11.2
Kopetdag	12.09 14:00	22.4	0.002	0.004	0.018	0	0	0.007	8.1	1.19	18.9	15.9
Kodj	16.09 17:00	22	0.042	0.026	0.018	0	0	0.005	8.2	1.06	14.9	14.1
Gazanjik	17.09 19:00	21.8	0.014	0.009	0.007	0	0	0.001	8.08	1.32	15.9	12.0

Table 5, (contd)

Reservoirs

Station	Date & time	T (°C)	Pesticide concentrations (μg l^{-1}) α-HCB	γ-HCB	β-HCB	DDT	DDD	DDE	O_2	BOD_5 (mg l^{-1})	COD	COD: BOD
Zeidskoe	25.08 11:00	23	0.001	0	0	0	0	0	8.85	1.3	10.7	8.2
Hanhovuz	04.09 08:00	22.8	0.004	0.001	0	0	0	0	9.8	1.6	27.9	17.4
Kopetdag-Skoe	11.09 16:00	22.6	0.003	0	0	0	0	0	7.81	1.06	9.3	8.8

Table 6. Changes in chemical parameters, in a body of water flowing along the Gara-Gum canal, 1996*

Station	Date & time	T (°C)	% O_2 sat	O_2	Total mins	Tot N (mg l^{-1})	COD	BOD_5
Golovnoye	05.08 10:50	25.7	108	8.74	718.0	1.168	37.6	0.93
2km	08.08 08:30	25.4	112	9.07	573.7	0.604	45.1	0.87
30km	10.08 16:30	29.2	140	10.57	690.5	0.955	33.8	0.76
Zahmet	11.08 09:20	25.6	112	9.01	637.4	0.655	56.4	0.83
Energetic	12.08 19:00	25.2	117	9.49	619.5	0.684	15.0	0.80
Mary	13.08 09:30	27.0	122	9.58	667.8	1.038	30.1	1.12
Tedjenstroy	13.08 12:20	27.5	124	9.67	569.2	1.035	18.8	1.11
Tedjen Bridge	13.08 15:30	27.7	120	9.42	970.8	1.184	86.5	1.10
Tedjen Dam	14.08 14:10	27.2	110	8.62	868.5	1.295	37.6	0.93
Dushak	17.08 07:30	26.8	113	8.92	619.2	0.853	30.1	0.92
Kaahka	18.08 07:30	27.5	105	8.23	676.2	1.092	52.6	1.10
Ashgabat	21.08 08:20	26.0	115	9.22	640.8	1.040	37.6	1.03
Pumping Stn	21.08 12:10	24.2	119	9.78	566.2	0.703	22.6	0.82
837km	21.08 19:20	24.5	114	9.37	597.6	0.544	71.4	0.78
Kopetdag	22.08 12:10	26.2	126	10.05	1040.4	0.857	56.4	1.03
Kodje	27.08 07:20	24.0	122	10.20	1081.1	1.592	45.1	0.82
Gazandjik	27.08 16:40	23.5	135	11.33	1326.0	1.692	45.1	1.14
Ave		26.1	118	9.49	756.6	0.999	42.5	0.95
(sd)		1.5	9	0.76	221.4	0.327	18.5	0.14

*Pesticides were only detected at a few stations and then only at trace levels.

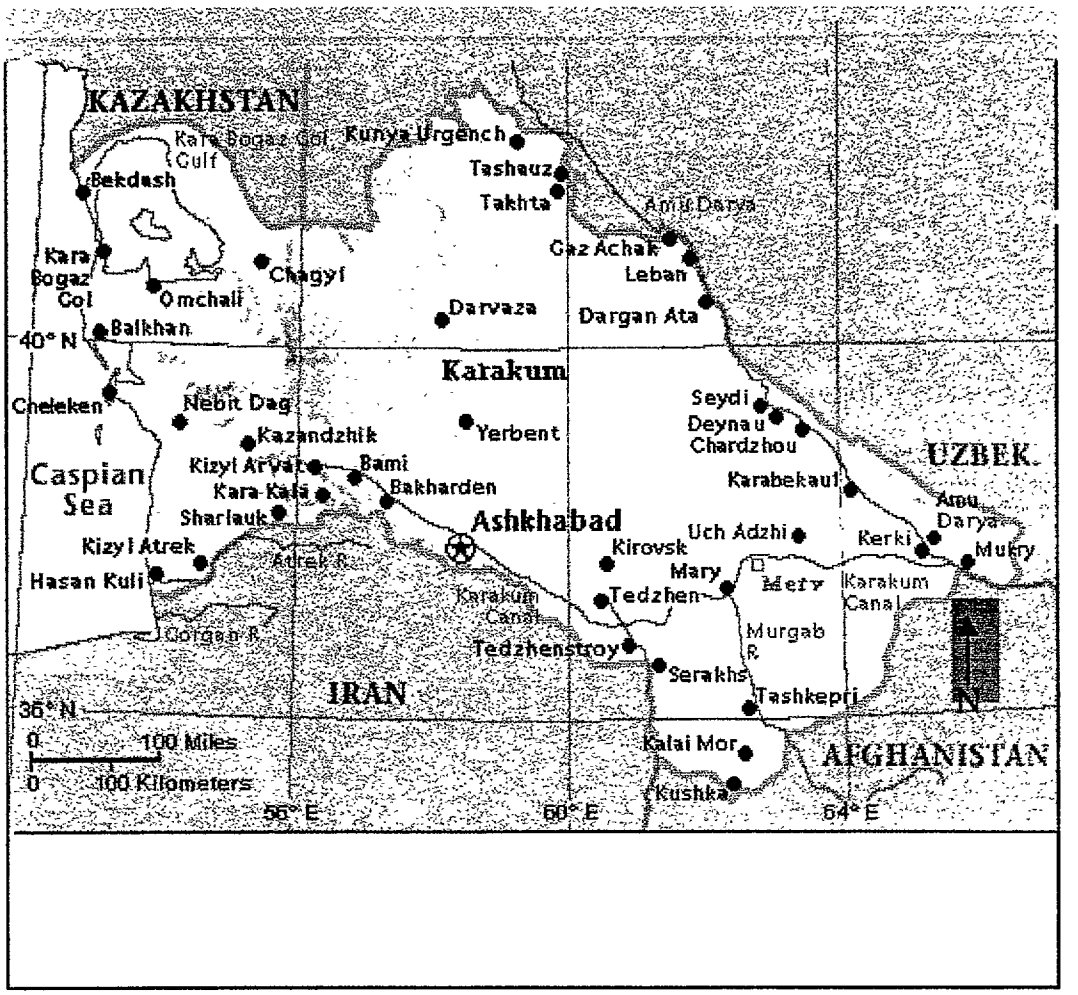
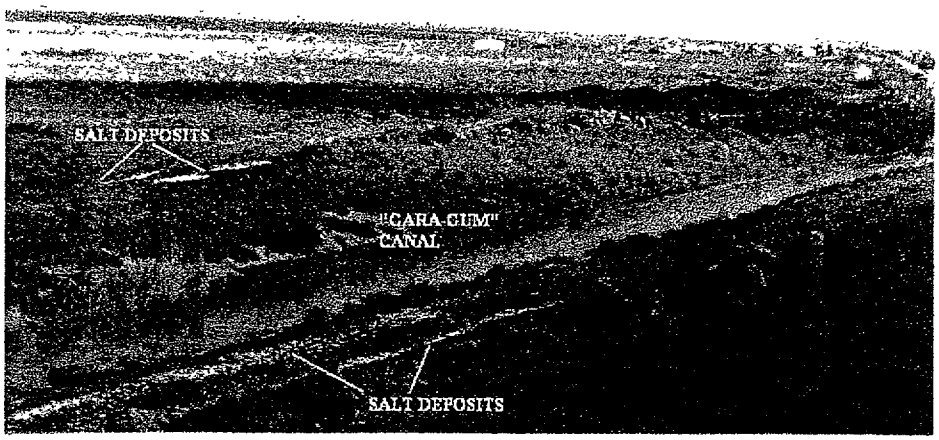

KAZAKHSTAN
Kara Bogaz Gol Gulf
Kunya Urgench
Tashauz
Takhta
Amu Darya
Bekdash
Kara Bogaz Gol
Chagyl
Omchali
Gaz Achak
Leban
Dargan Ata
Darvaza
Balkhan
40° N
Karakum
Cheleken
Nebit Dag
Kazandzhik
Yerbent
Seydi
Deynau
Chardzhou
UZBEK.
Caspian Sea
Kizyl Arvat
Bami
Kara-Kala
Bakharden
Karabekaul
Sharlauk
Ashkhabad
Uch Adzhi
Kerki
Amu Darya
Mukry
Kizyl Atrek
Atrek R.
Kirovsk
Mary
Mery
Karakum Canal
Hasan Kuli
Karakum Canal
Tedzhen
Murgab R.
Gorgan R.
Tedzhenstroy
Serakhs
IRAN
Tashkepri
N
36° N
0 100 Miles
0 100 Kilometers
Kalai Mor
AFGHANISTAN
Kushka
56° E
60° E
64° E

Fig. 1


SALT DEPOSITS
"CARA-GUM"
CANAL
SALT DEPOSITS

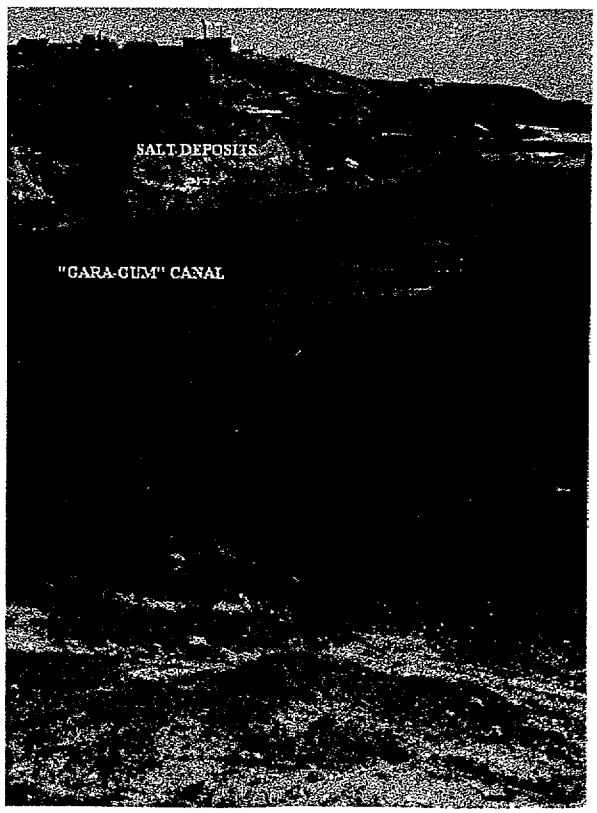
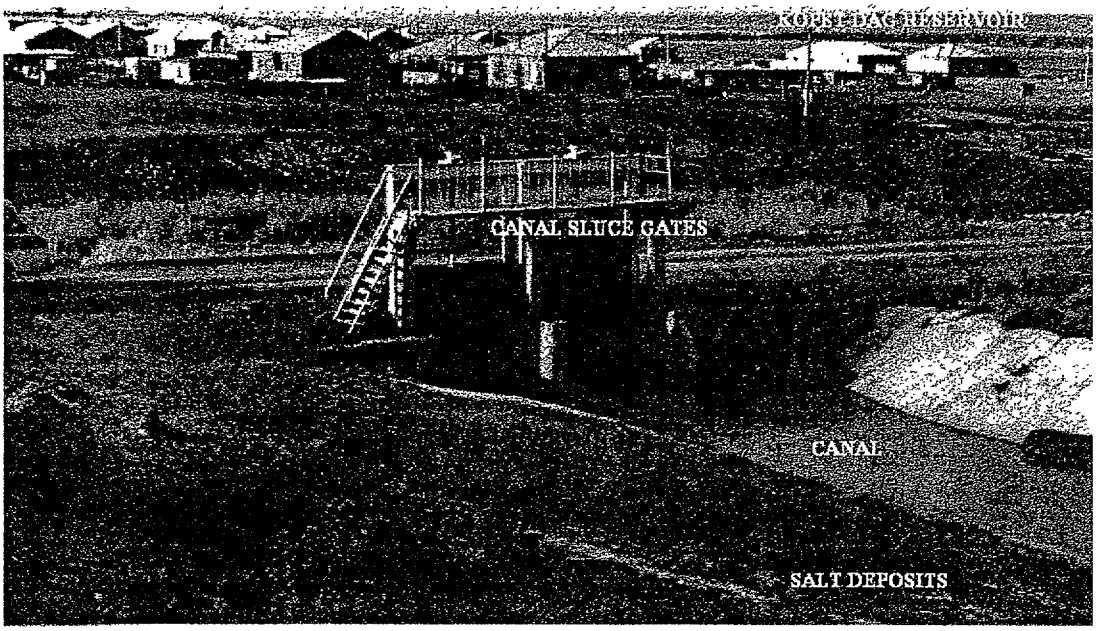

SALT DEPOSITS
"CARA-GUM" CANAL

Fig. 2



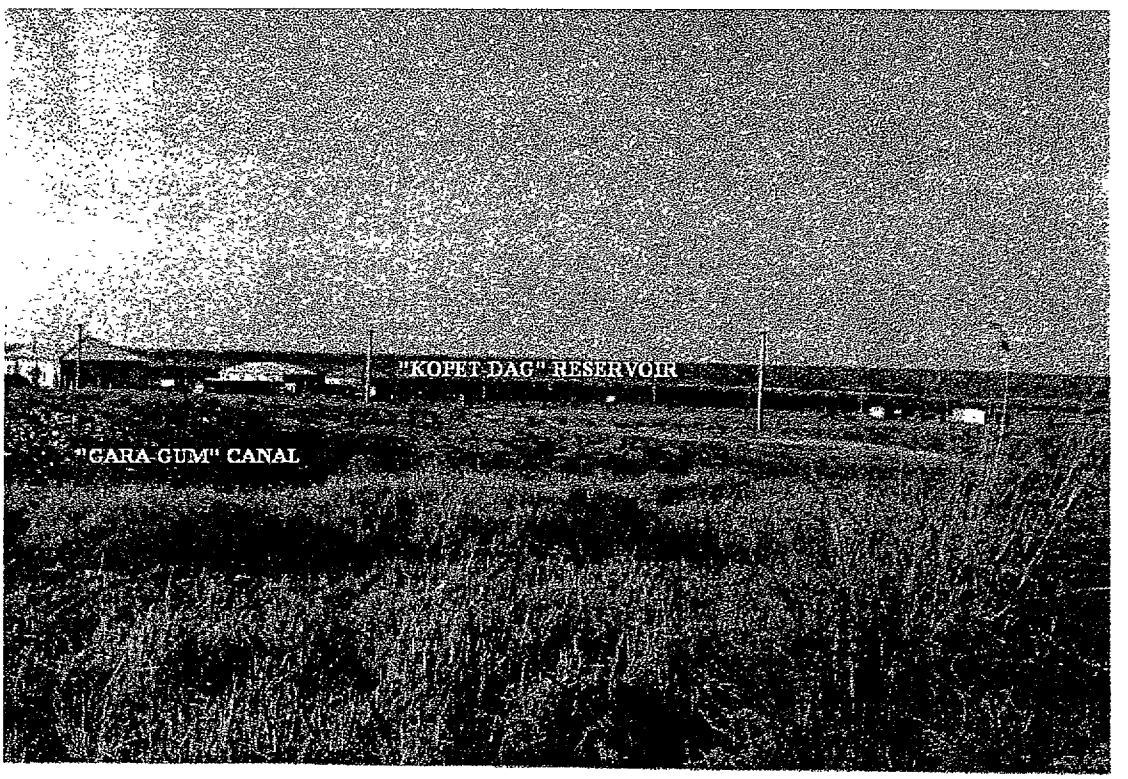
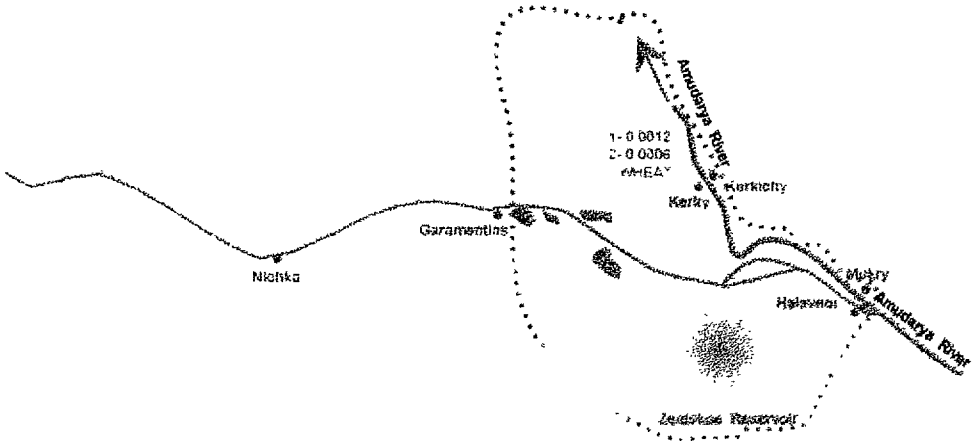


Fig. 2 continued

A


Amudarya River
1- 0.0012
2- 0.0006
WHEAT
Kerkichy
Kerky
Garamentlas
Nichka
Mukry
Halaveni
Amudarya River
Zeidskoe Reservoir

B

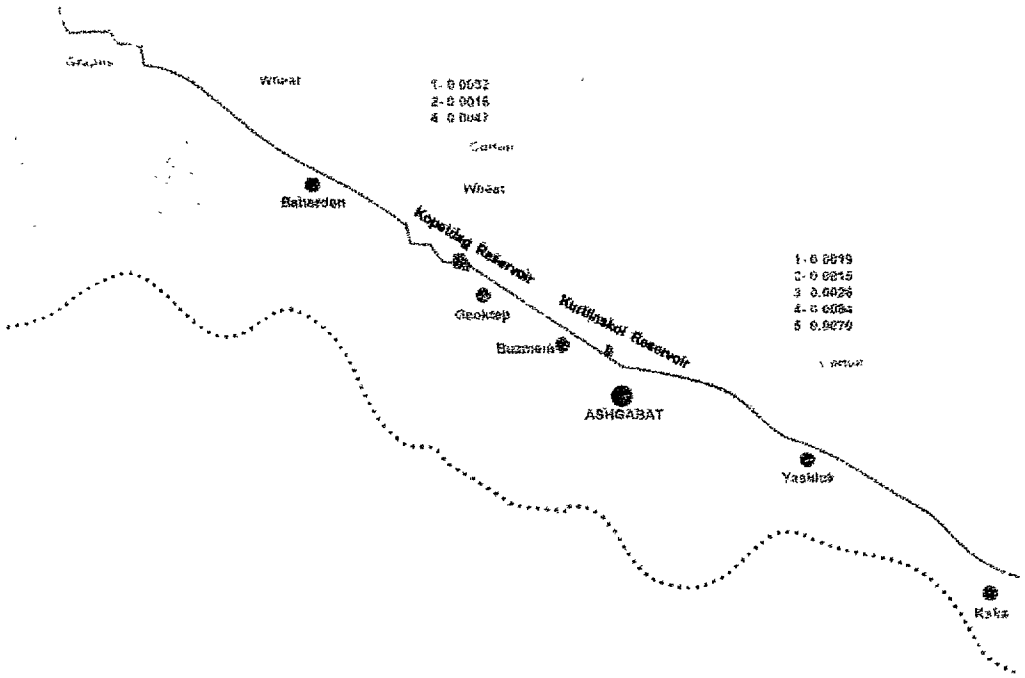
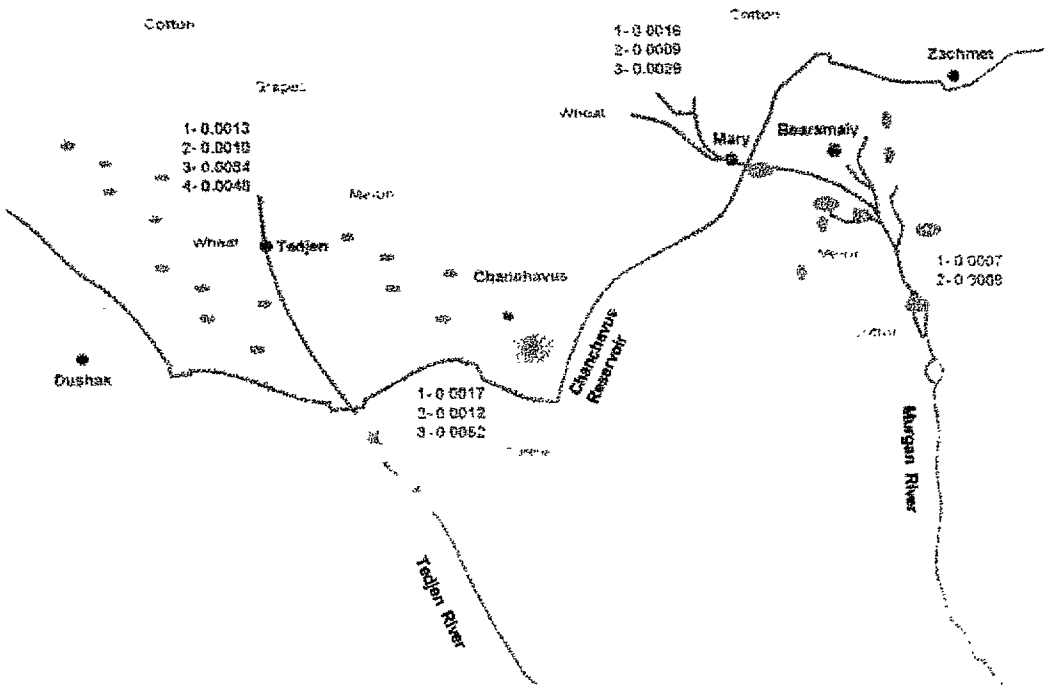

Wheat
1- 0.0032
2- 0.0016
4- 0.0047
Wheat
Baharden
Kopetdag Reservoir
Geoktep
Kurtlinskoi Reservoir
Buzmein
ASHGABAT
Yashlok
Kaka

Fig. 3

C



D

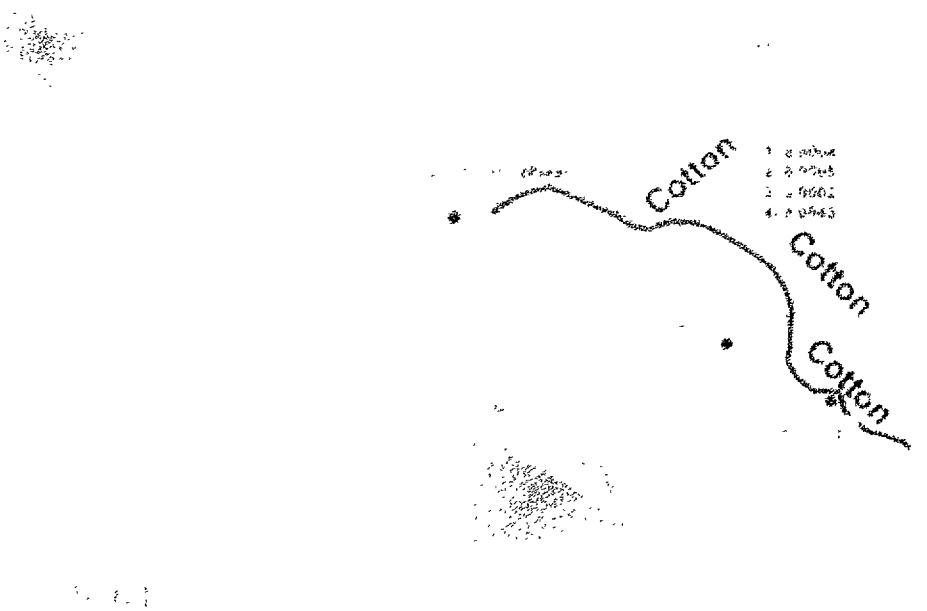
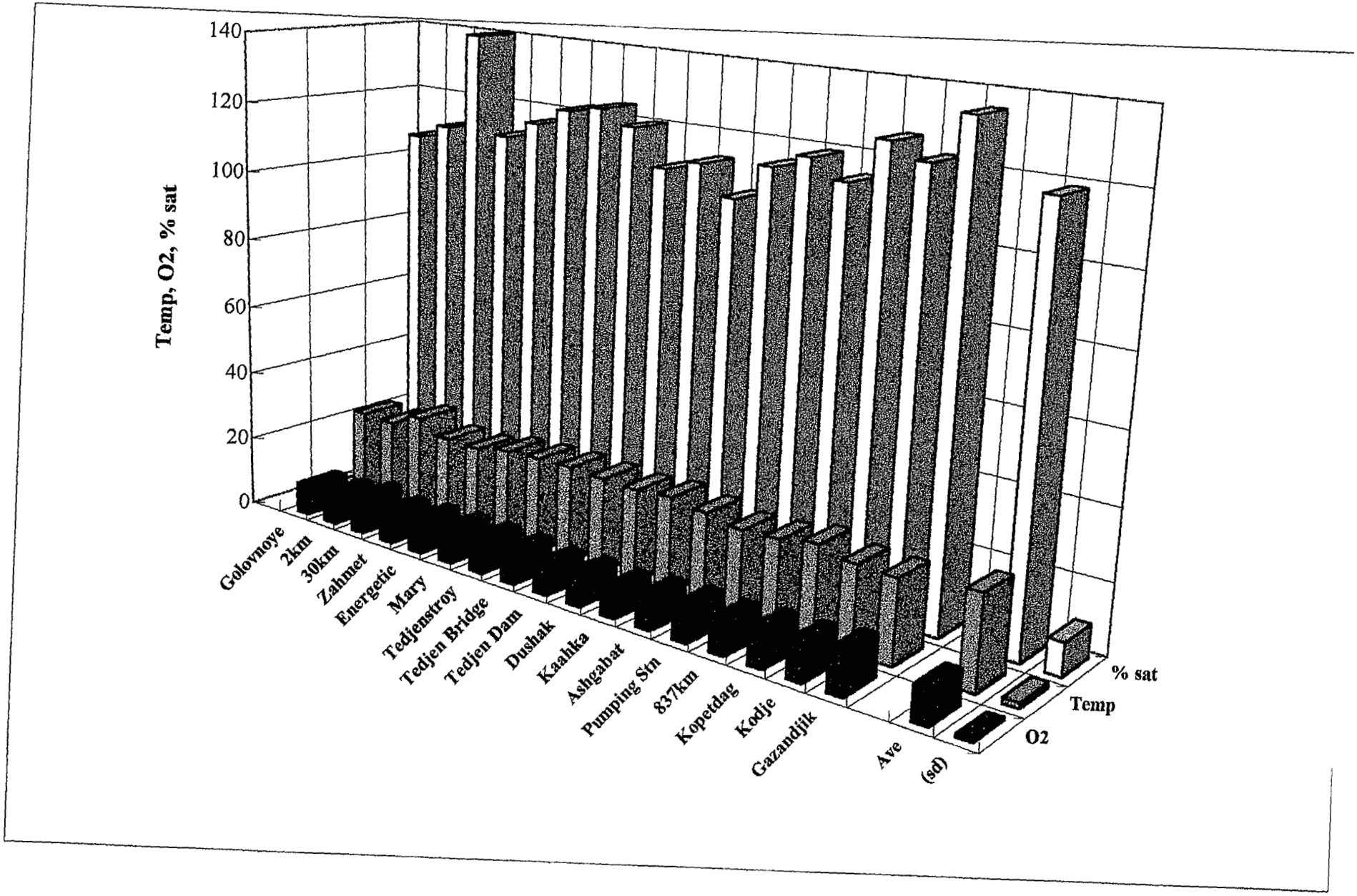


Fig.3 continued


Temp, O2, % sat
140
120
100
80
60
40
20
0
Golovnoye
2km
30km
Zahmet
Energetic
Mary
Tedjenstroy
Tedjen Bridge
Tedjen Dam
Dushak
Kaahka
Ashgabat
Pumping Stn
837km
Kopetdag
Kodje
Gazandjik
Ave
(sd)
% sat
Temp
O2

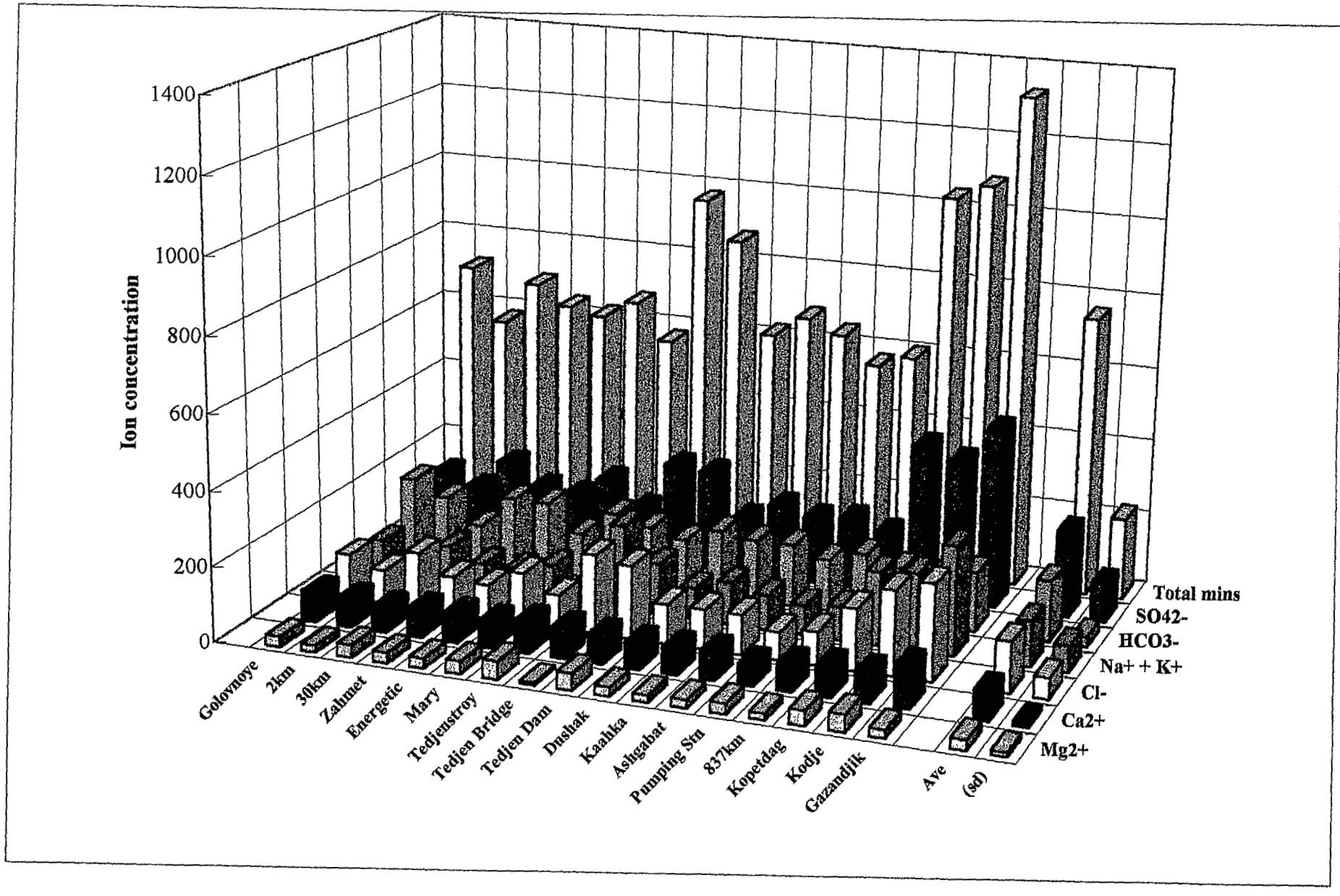

Ion concentration
1400
1200
1000
800
600
400
200
0
Golovnoye
2km
30km
Zahmet
Energetic
Mary
Tedjenstroy
Tedjen Bridge
Tedjen Dam
Dushak
Kaahka
Ashgabat
Pumping Stn
837km
Kopetdag
Kodje
Gazandjik
Ave
(sd)
Total mins
SO42-
HCO3-
Na+ + K+
Cl-
Ca2+
Mg2+

Fig.5

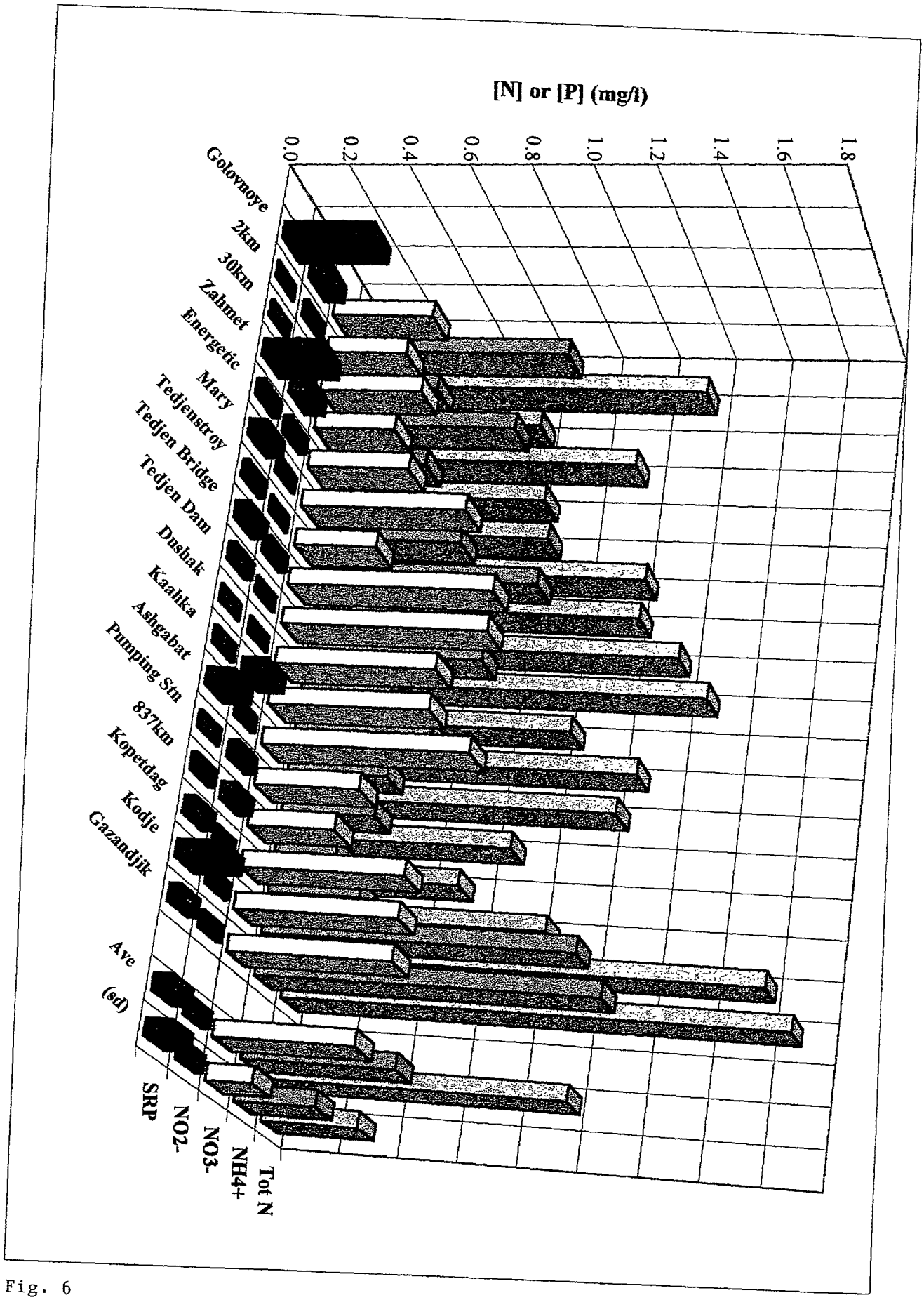
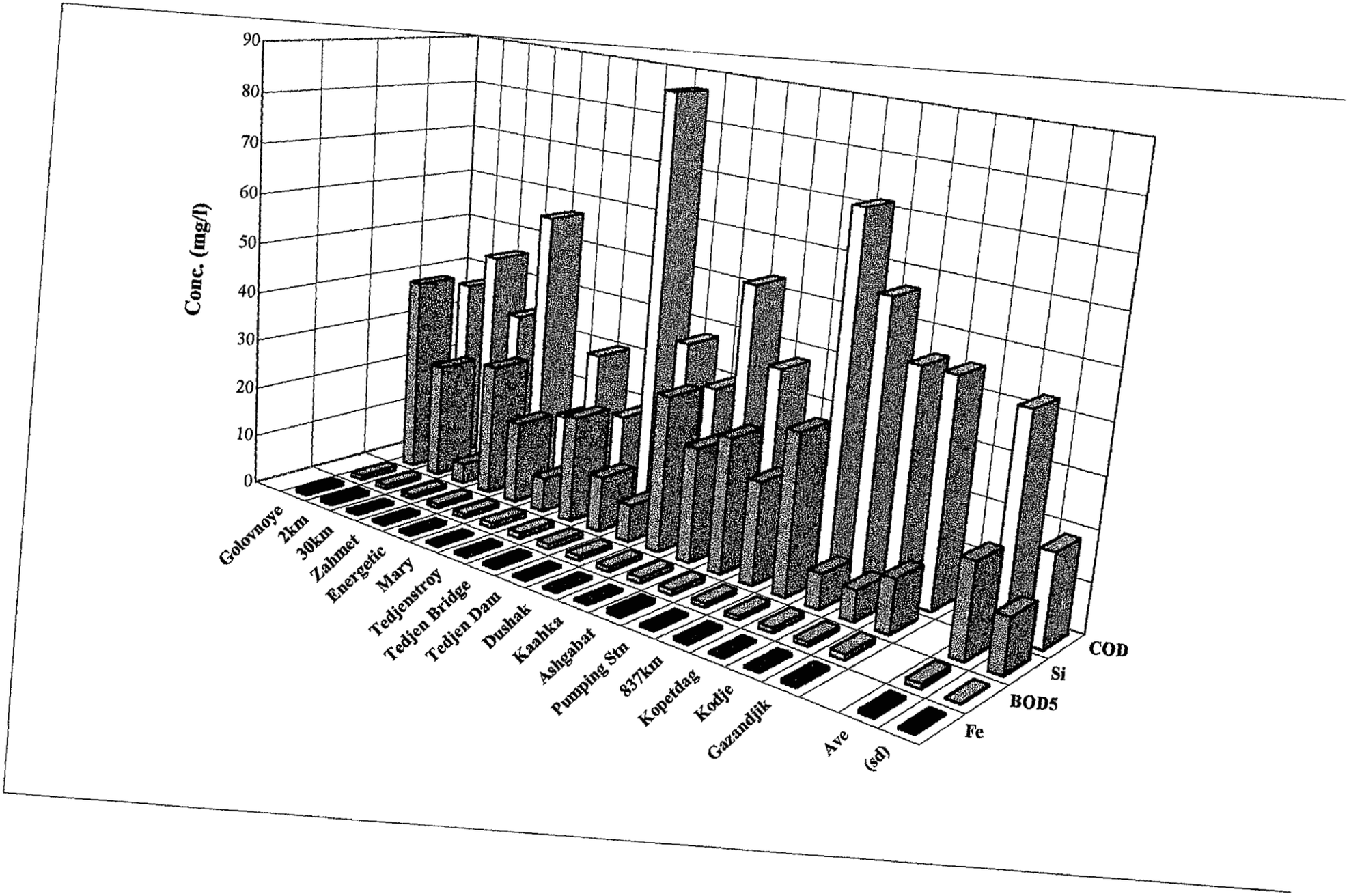

[N] or [P] (mg/l)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Golovnoye
2km
30km
Zahmet
Energetic
Mary
Tedjenstroy
Tedjen Bridge
Tedjen Dam
Dushak
Kaahka
Ashgabat
Pumping Stn
837km
Kopetdag
Kodje
Gazandjik
Ave
(sd)
SRP
NO2-
NO3-
NH4+
Tot N

Fig. 6


Conc. (mg/l)
90
80
70
60
50
40
30
20
10
0
Golovnoye
2km
30km
Zahmet
Energetic
Mary
Tedjenstroy
Tedjen Bridge
Tedjen Dam
Dushak
Kaahka
Ashgabat
Pumping Stn
837km
Kopetdag
Kodje
Gazandjik
Ave
(sd)
COD
Si
BOD5
Fe

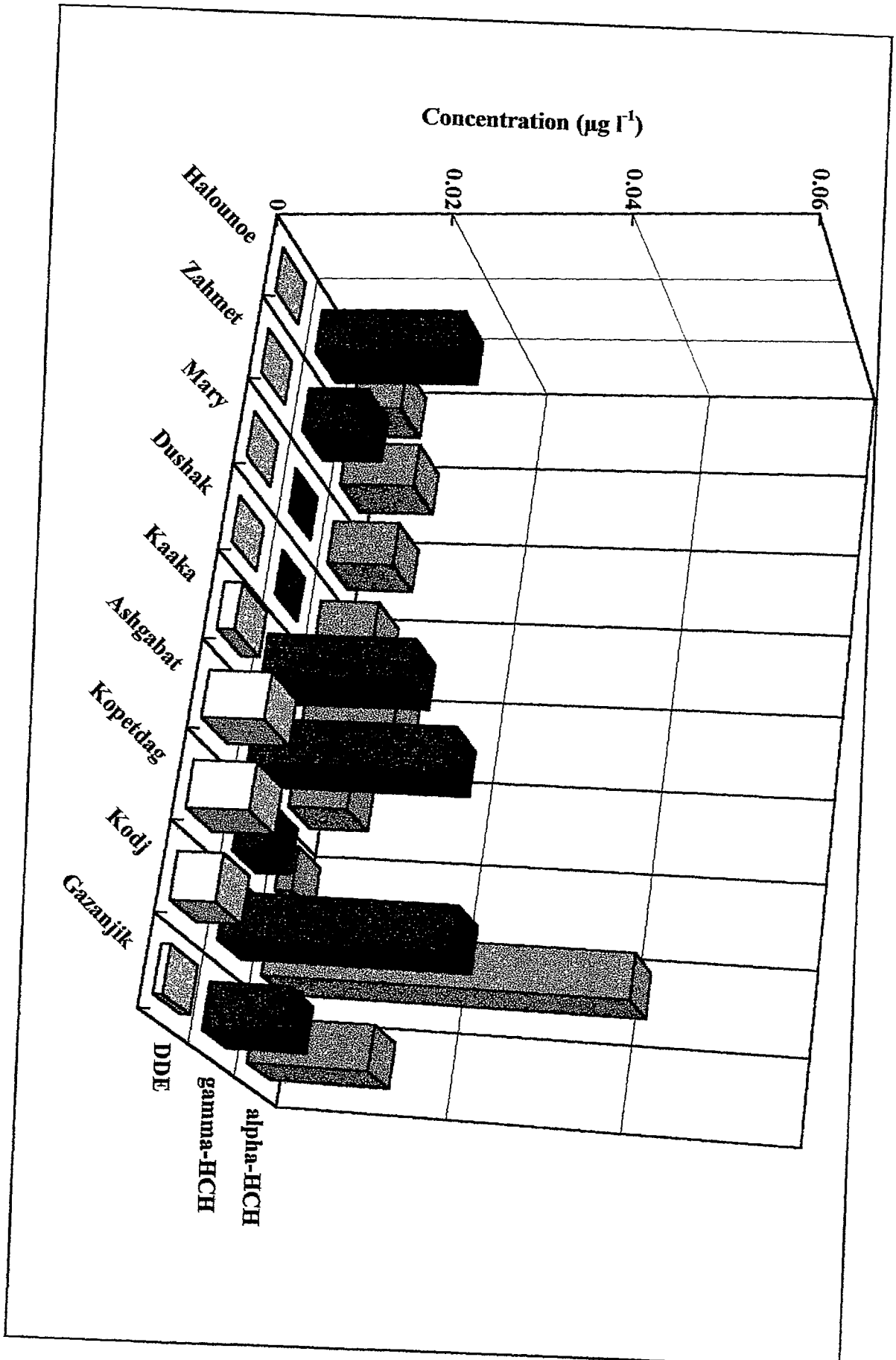

0.06
0.04
0.02
0
Concentration (µg l-1)
alpha-HCH
gamma-HCH
DDE
Halounoe
Zahmet
Mary
Dushak
Kaaka
Ashgabat
Kopetdag
Kodj
Gazanjik

Fig 8

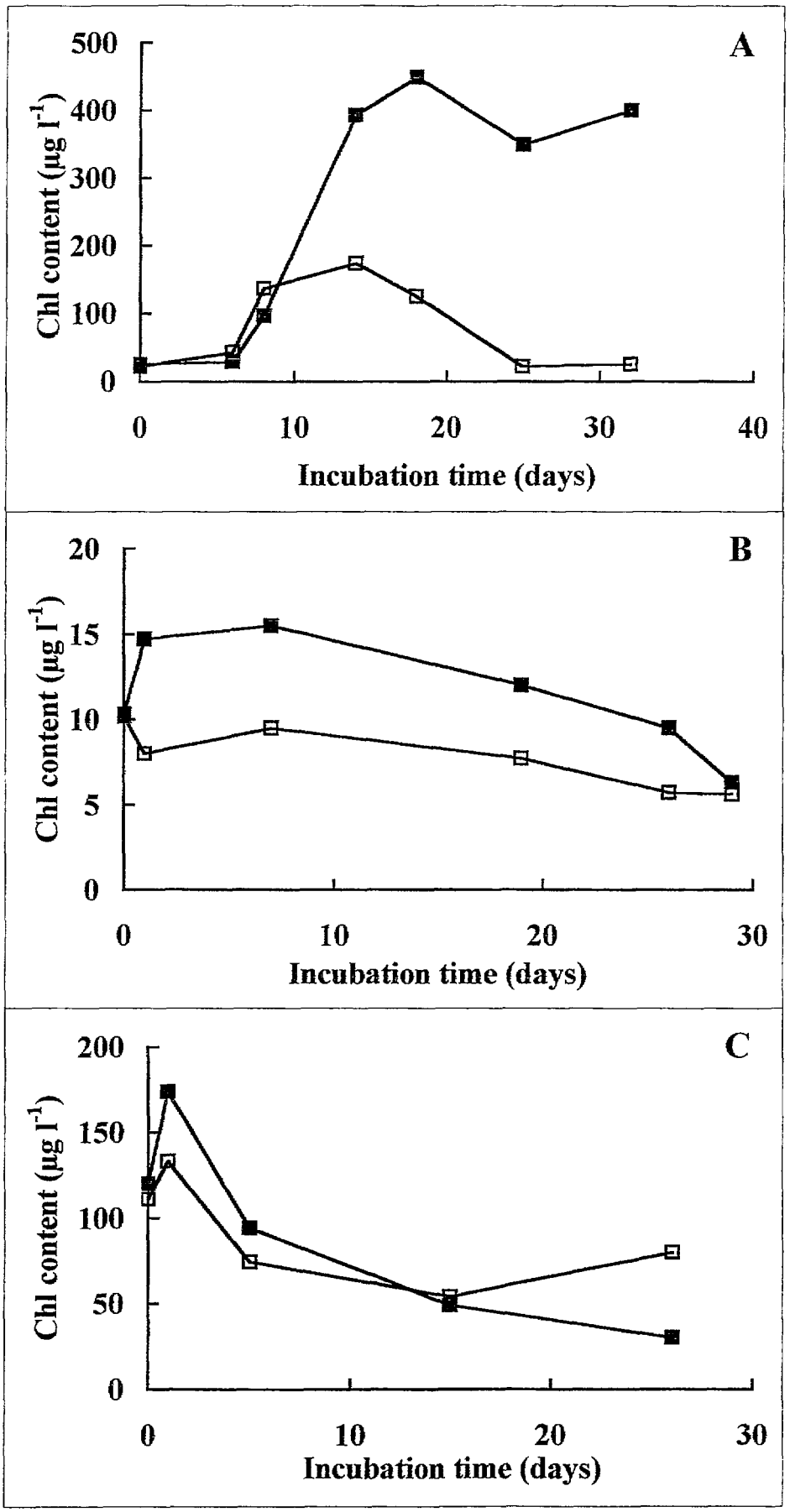

A
500
400
300
200
100
0
Chl content (µg l-1)
0
10
20
30
40
Incubation time (days)
B
20
15
10
5
0
Chl content (µg l-1)
0
10
20
30
Incubation time (days)
C
200
150
100
50
0
Chl content (µg l-1)
0
10
20
30
Incubation time (days)

Fig. 9

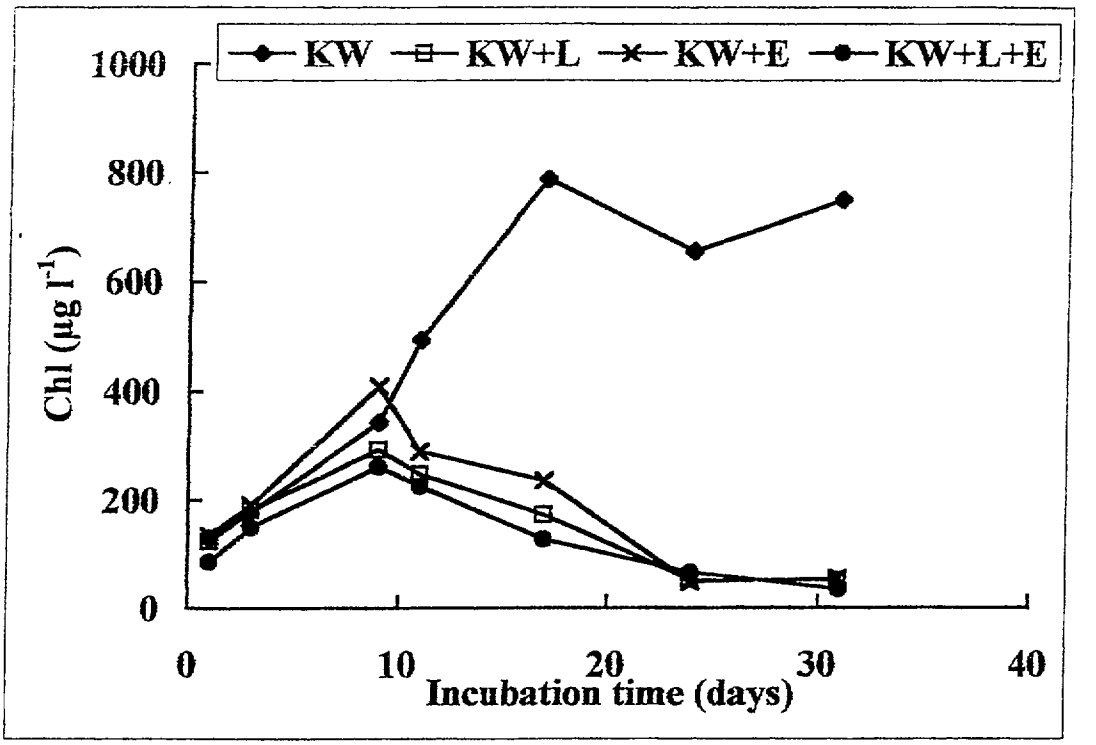
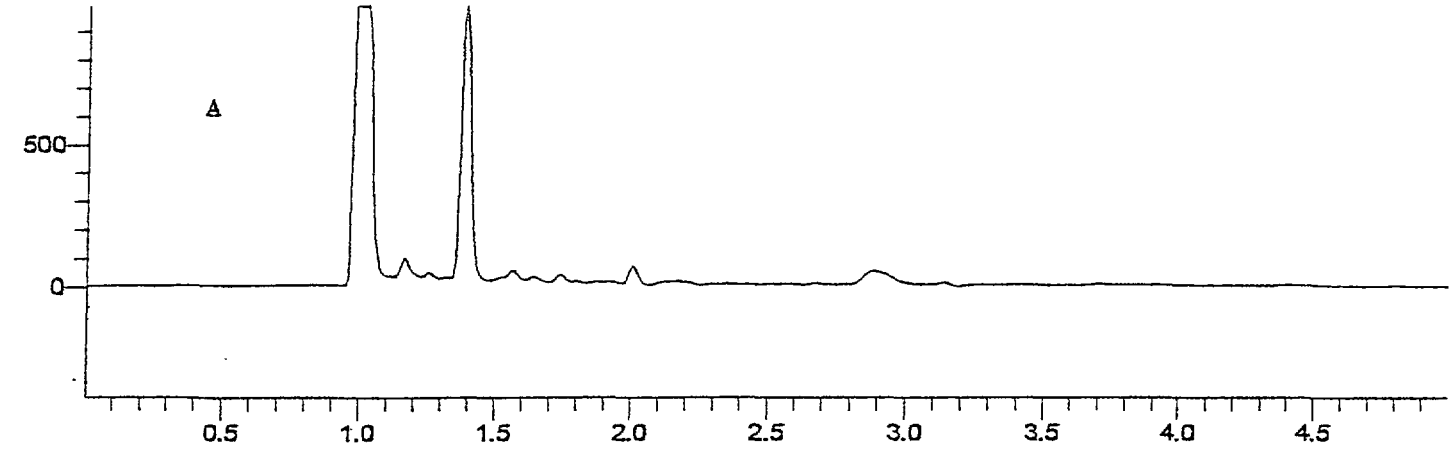
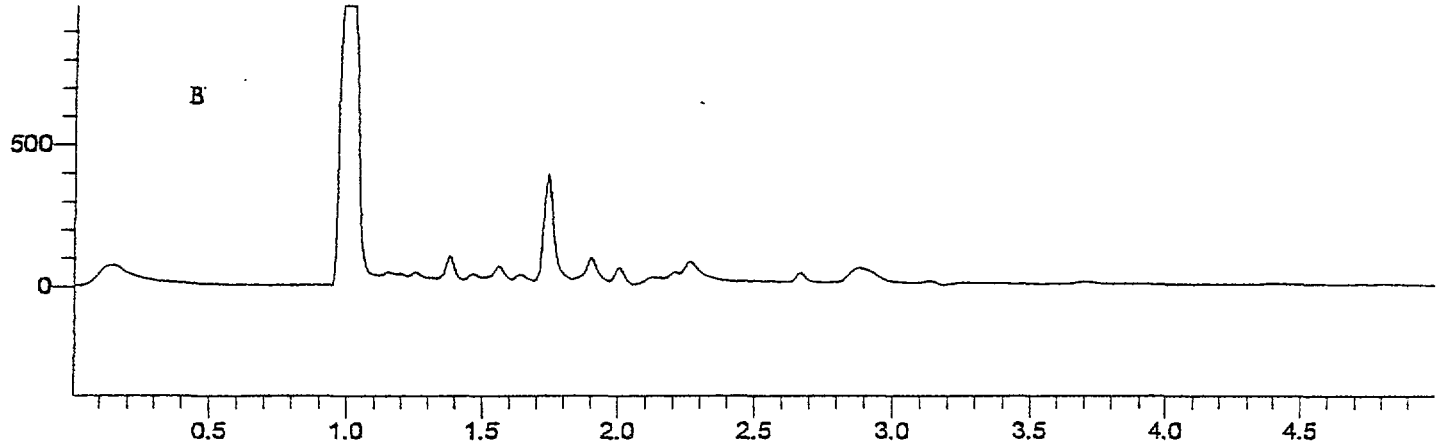
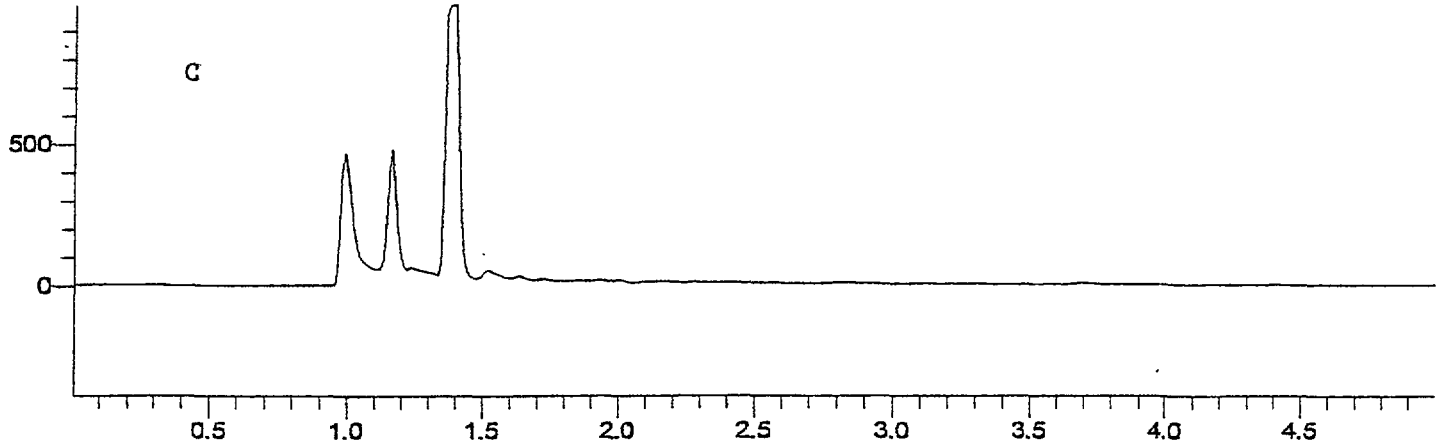
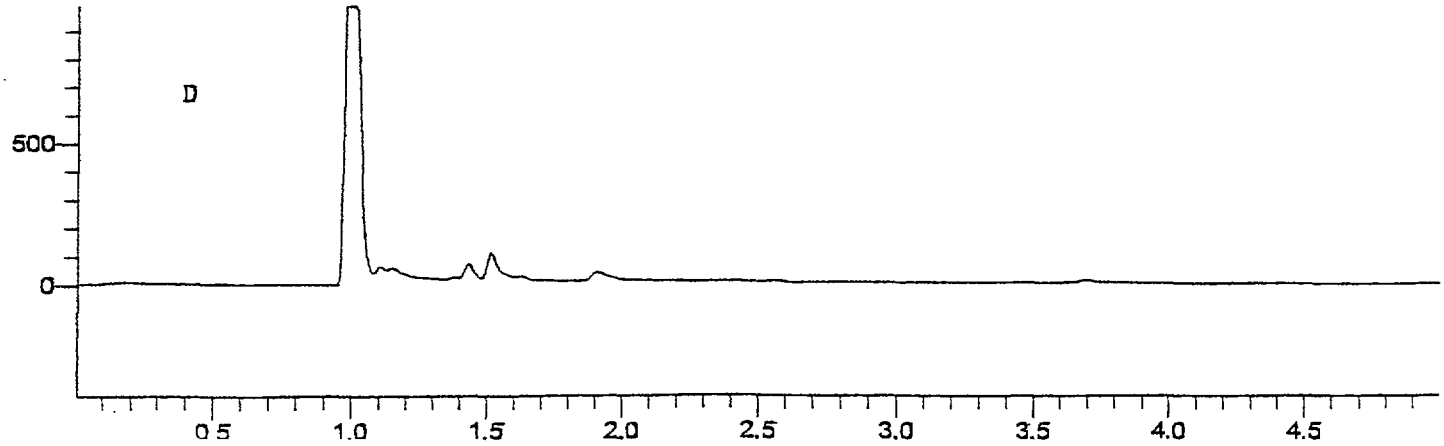

KW
KW+L
KW+E
KW+L+E
1000
800
600
400
200
0
Chl (µg l-1)
0
10
20
30
40
Incubation time (days)

Fig. 10


A
500
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5


B
500
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5


C
500
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5


D
500
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5

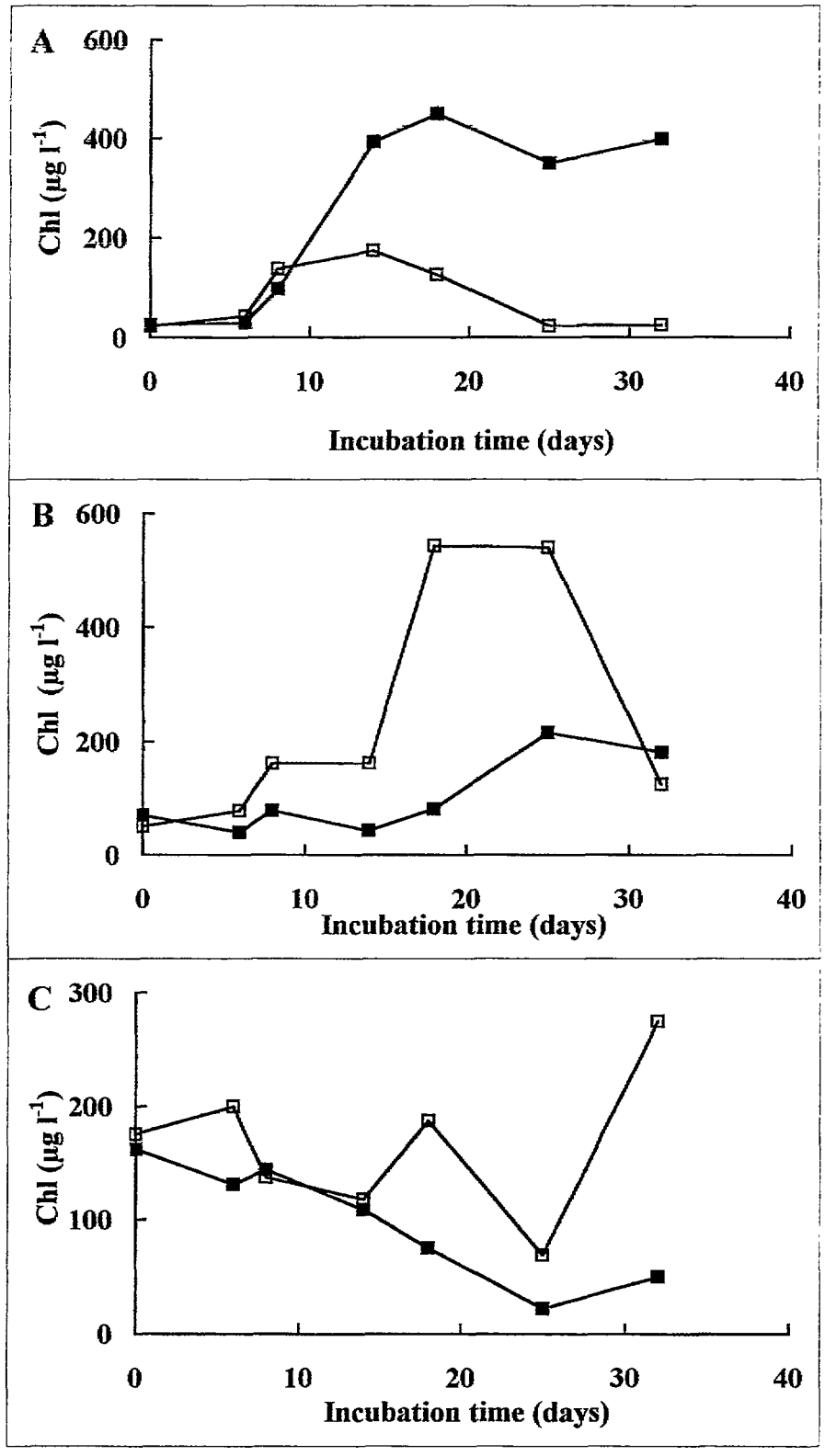

A
600
400
200
0
Chl (µg l-1)
0
10
20
30
40
Incubation time (days)
B
600
400
200
0
Chl (µg l-1)
0
10
20
30
40
Incubation time (days)
C
300
200
100
0
Chl (µg l-1)
0
10
20
30
40
Incubation time (days)

Fig.12

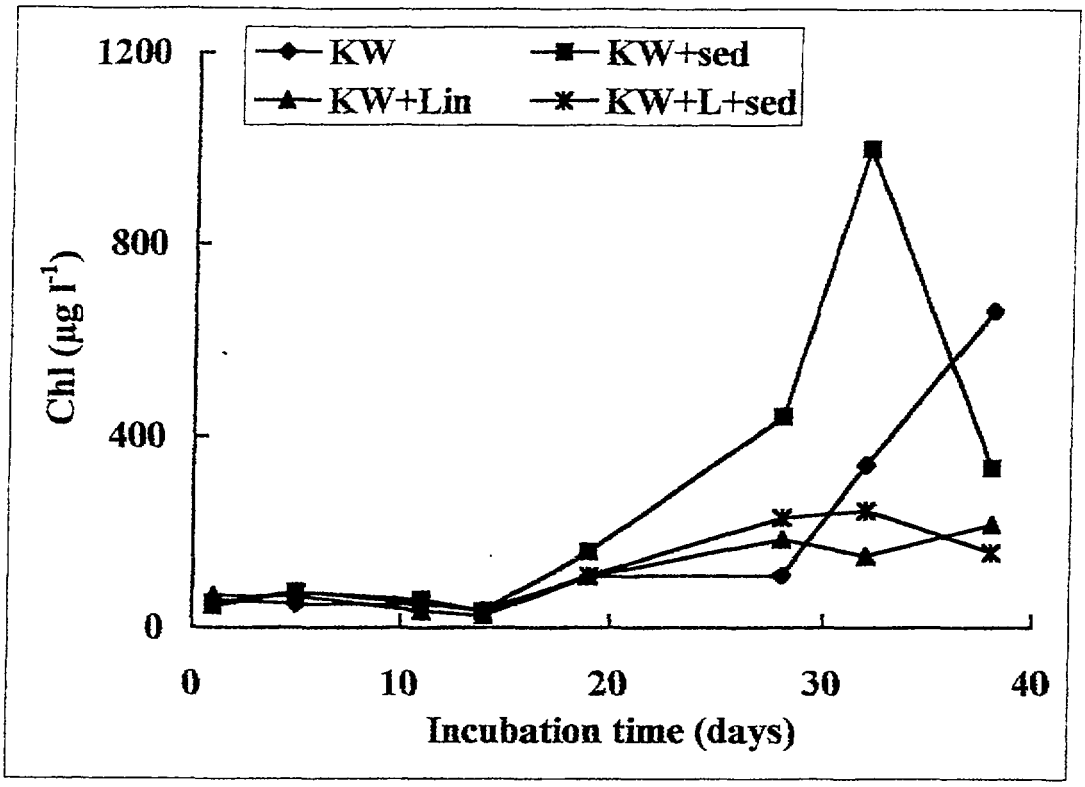

KW
KW+sed
KW+Lin
KW+L+sed
1200
800
400
0
Chl (µg l-1)
0
10
20
30
40
Incubation time (days)

Fig. 13

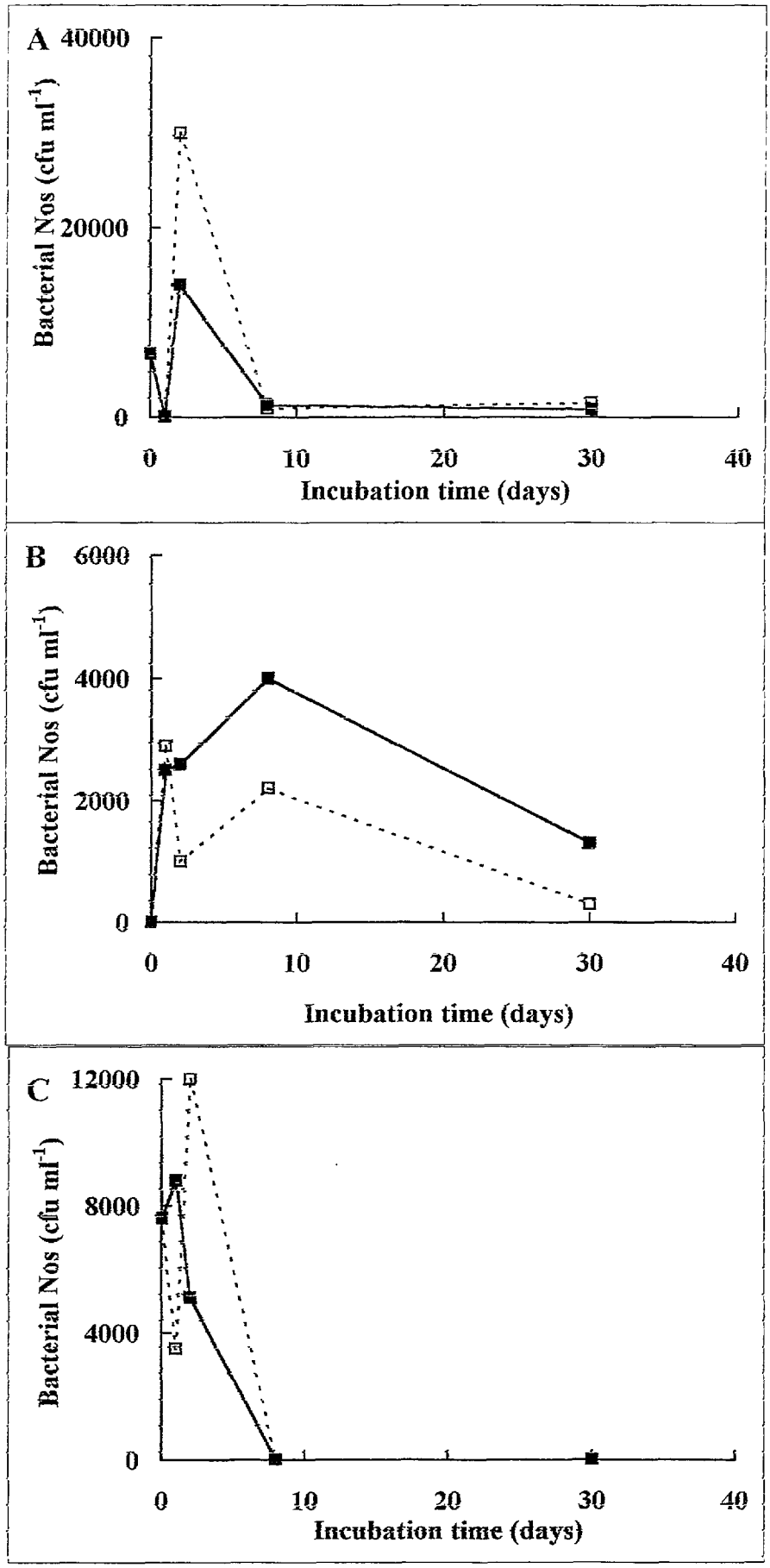

A
40000
20000
0
Bacterial Nos (cfu ml-1)
0
10
20
30
40
Incubation time (days)
B
6000
4000
2000
0
Bacterial Nos (cfu ml-1)
0
10
20
30
40
Incubation time (days)
C
12000
8000
4000
0
Bacterial Nos (cfu ml-1)
0
10
20
30
40
Incubation time (days)

Fig. 14

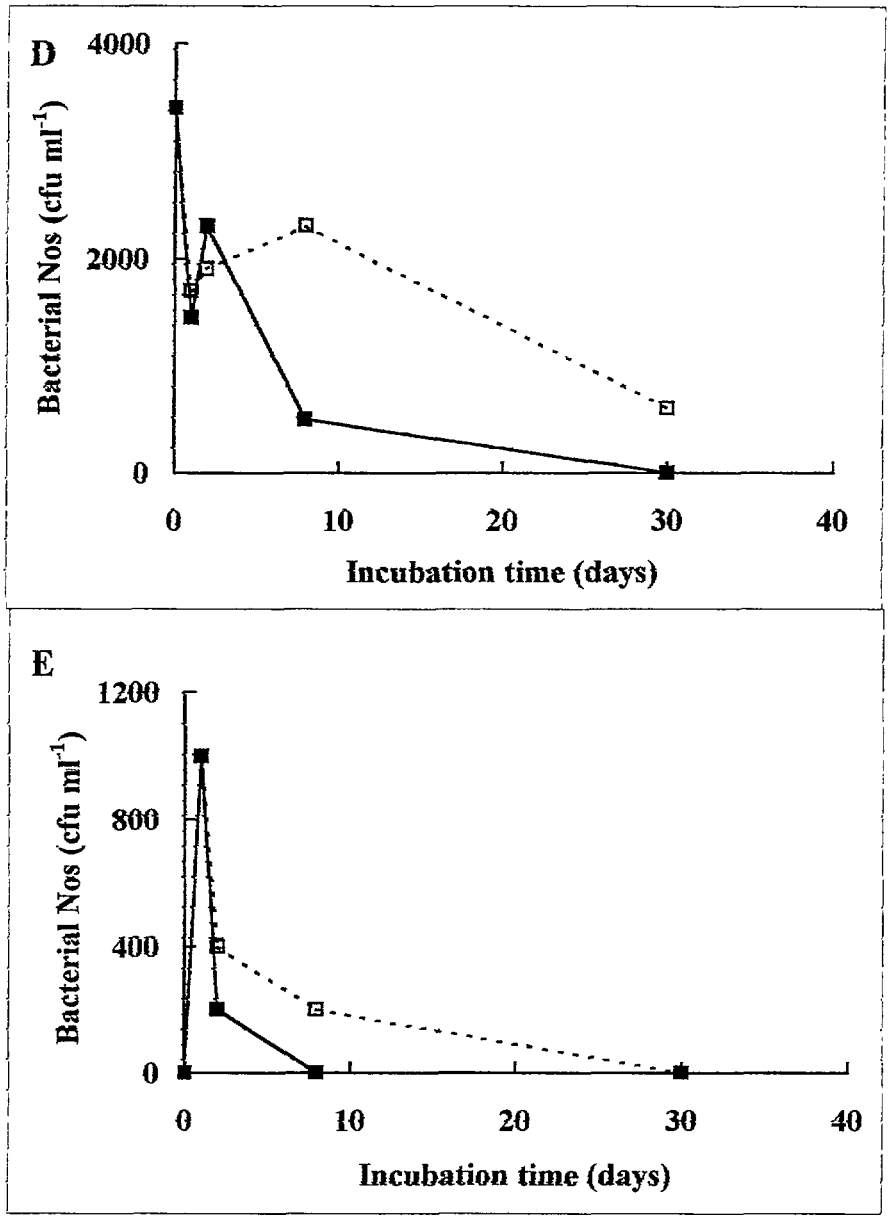


Fig. 14 continued

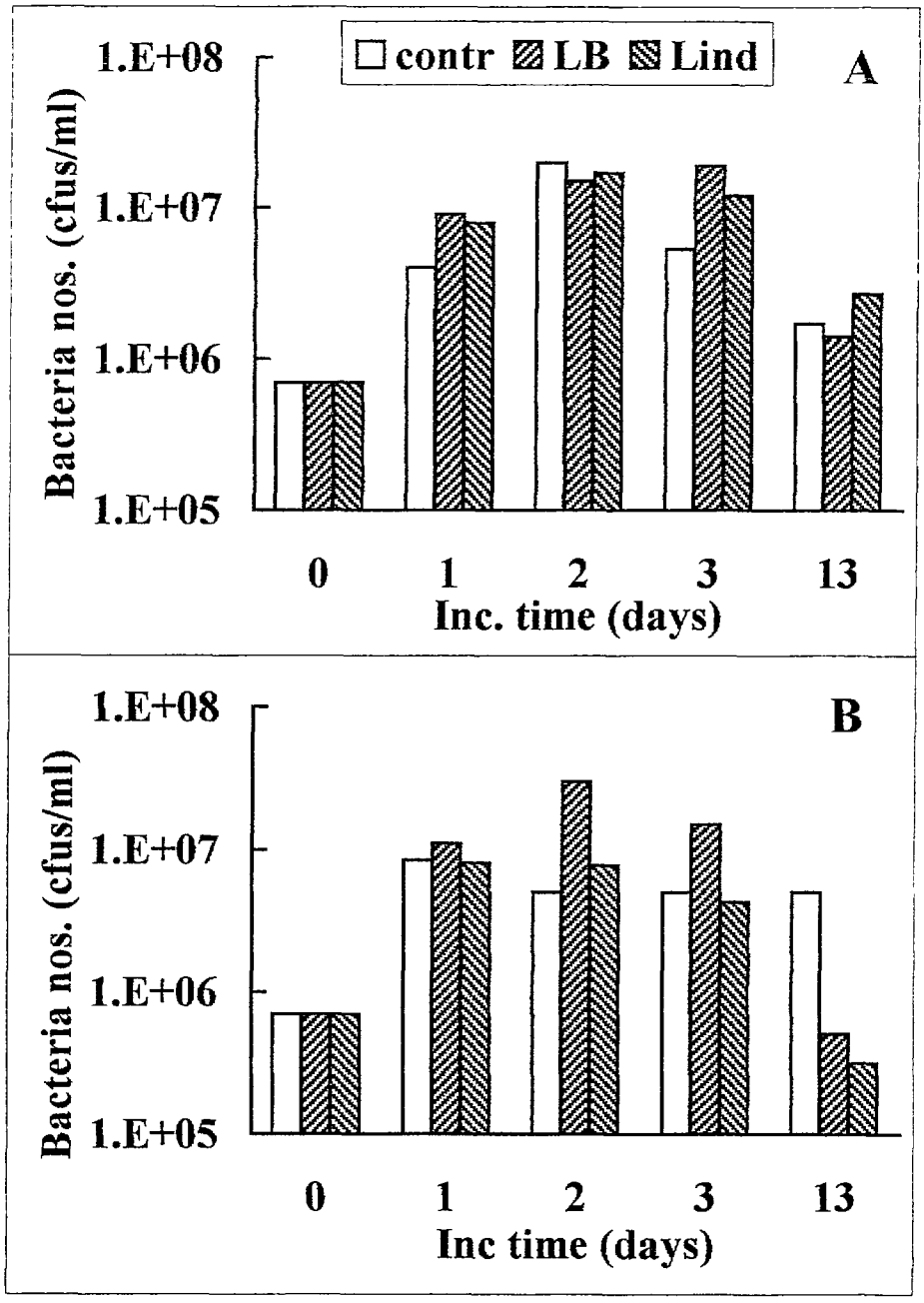
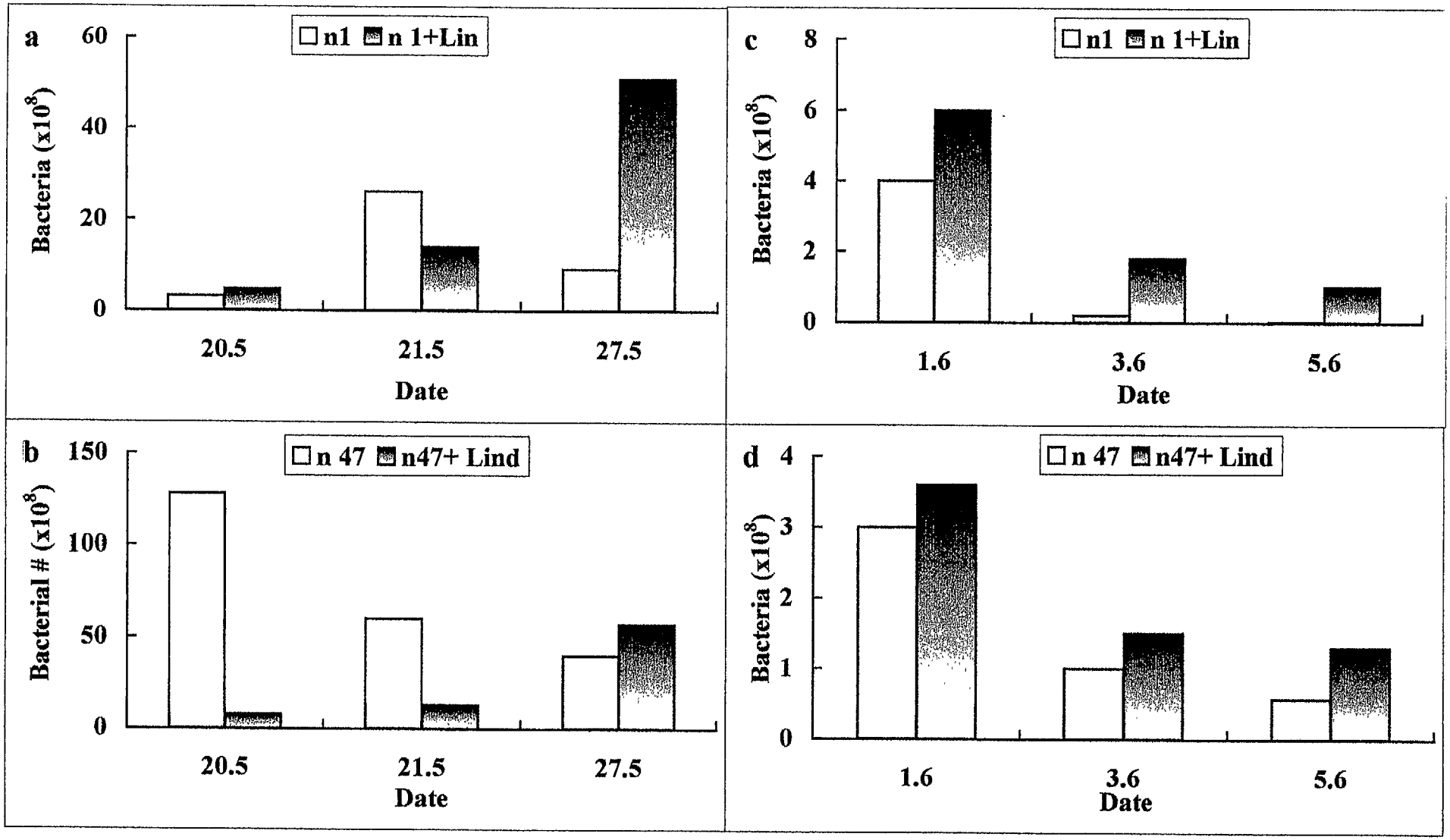

contr
LB
Lind
A
1.E+08
1.E+07
1.E+06
1.E+05
Bacteria nos. (cfus/ml)
0
1
2
3
13
Inc. time (days)
B
1.E+08
1.E+07
1.E+06
1.E+05
Bacteria nos. (cfus/ml)
0
1
2
3
13
Inc time (days)

Fig. 15


a
n1
n 1+Lin
Bacteria (x10^8)
60
40
20
0
20.5
21.5
27.5
Date
b
n 47
n47+ Lind
Bacterial # (x10^8)
150
100
50
0
20.5
21.5
27.5
Date
c
n1
n 1+Lin
Bacteria (x10^8)
8
6
4
2
0
1.6
3.6
5.6
Date
d
n 47
n47+ Lind
Bacteria (x10^8)
4
3
2
1
0
1.6
3.6
5.6
Date

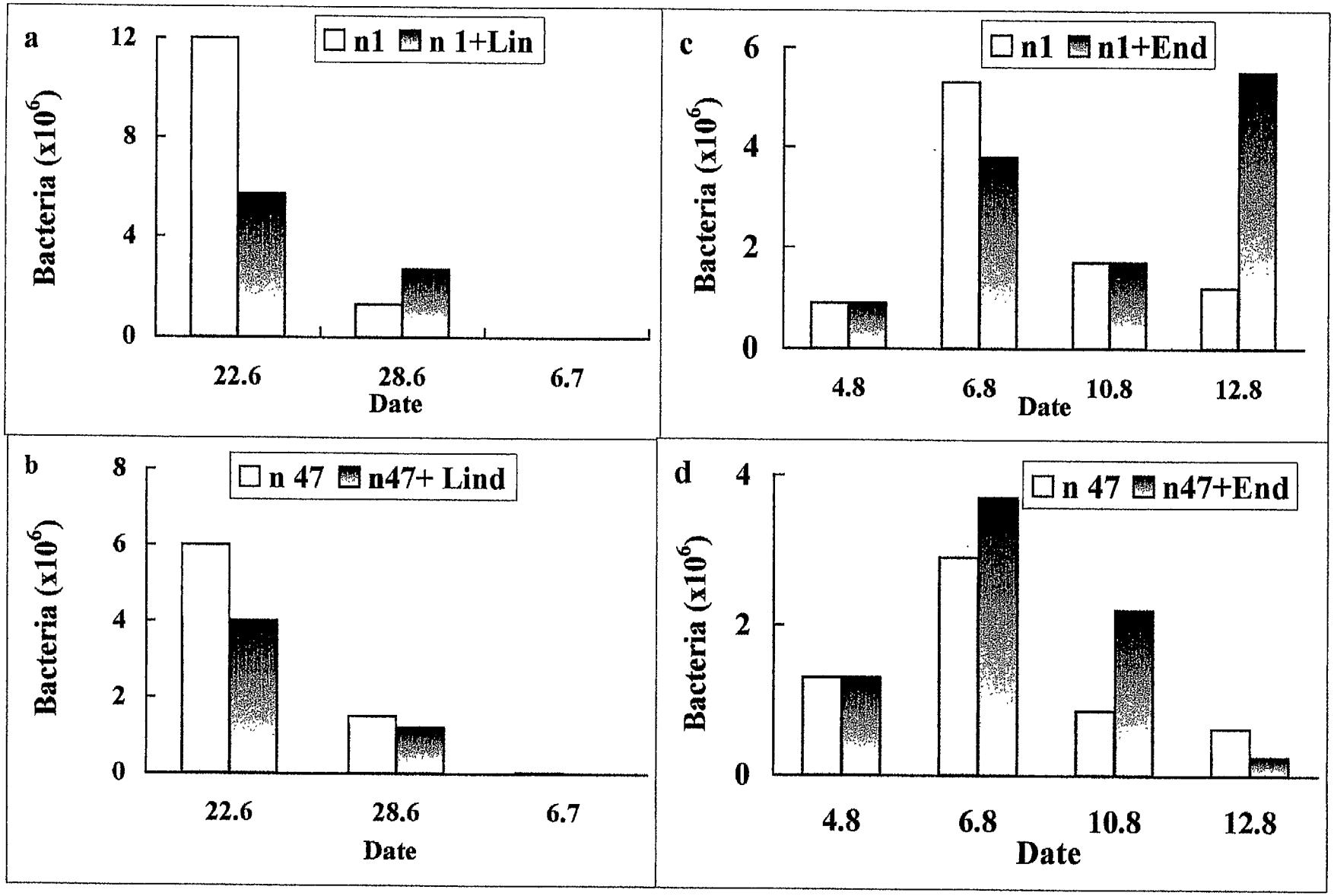

a
n1
n 1+Lin
Bacteria (x10^6)
12
8
4
0
22.6
28.6
6.7
Date
b
n 47
n47+ Lind
Bacteria (x10^6)
8
6
4
2
0
22.6
28.6
6.7
Date
c
n1
n1+End
Bacteria (x10^6)
6
4
2
0
4.8
6.8
10.8
12.8
Date
d
n 47
n47+End
Bacteria (x10^6)
4
2
0
4.8
6.8
10.8
12.8
Date

Fig. 17