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Utilization of Ostracods (Crustacea) as Bioindicator for Environmental Pollutants

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Authors' contributions

This work was carried out in collaboration among all authors. Author EP *designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors* VD *and* RK *managed the analyses of the study. Authors* TI and SA *managed the literature searches. All authors read and approved the final manuscript.*

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ABSTRACT

Ecosystem undergoes drastic changes due to the anthropogenic activities. As a consequence of industrial development, increasing population growth and modernized agricultural practices water resources like limnetic zone and marine areas have undergone eutrophication. This resulted in the decline in population of phytoplankton and zooplankton. Hence, it is an urgent need to monitor the quality of the environment. Several organisms are used as biomonitors. Among them, Ostracodes (Seed Shrimps) which belong to Crustacean group are very sensitive to those changes in the environment and useful in predicting the paleo environmental conditions. Ostracodes are bivalve arthropods which are enclosed in a carapace made of low magnesium calcite. These species are occurring for about 450 million years dates back to ordovician which are known for their easier fossilization. The development of Ostracodes is influenced by the physic - chemical properties of

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waters such as Salinity, temperature, pH, Dissolved oxygen, bottom grain sizes and sedimentation rates. In addition to diversity and abundance of population, morphological and geochemical changes can also be detected in the Ostracod carapace (shell) which serves as a tracer of the water quality. These details are basis for utilizing Ostracods as paleoenvironmental (paleoclimatic, paleosalinity, paleooceanographic) reconstruction, ecotoxicity monitoring, biostratigraphic indicator. Moreover, these microcrustaceans showed similar or higher sensitivity to herbicides, pesticides, oil spills or heavy metals pollution other than traditional groups like copepods, protozoan, rotifers, cladocerans which are used to test the human impacts on ecosystem. These meiofaunas are highly adaptable to waters containing organic and inorganic contaminants generated by catastrophic activities by human beings in the surroundings.

Keywords: Ostracods; bioindicatiors; environment; pollutants.

1. INTRODUCTION

Increasing urbanization has lead to the decline of fresh water resources as well as marine and coastal areas over the last two centuries. Industrial, agricultural and technological activities have triggered changes in the environment. Industrial revolution, increasing population growth and modernized agricultural practices have resulted in the increased nutrient loading in lakes and rivers and change of freshwater drainage supply basins. Rivers are severely affected by the anthropogenic activities that reach the coastal ecosystems affecting water quality and amplifying the eutrophication. As a result decreasing oxygen level in the water column have created impact on the marine flora and fauna.

In order to judge these impacts, several organisms have been used as bioindicators. These involve meio faunal species like Arthropods. Major part of them are Crustaceans like lobsters, crayfish, shrimp, copepods, ostracods. Among them Ostracods (Ostracodes) are more responsive to the physio-chemical conditions and their carapace morphology. Hence they are used as bio indicators and inaddition they are used in paleoenvironmental (paleoclimatic, paleosalinity, paleooceanographic) reconstruction, ecotoxicity monitoring, biostratigraphic indicator. Moreover, these microcrustaceans exhibited similar or increased sensitivity to pesticides, herbicides, heavy metal or oil spills pollution other than traditional groups like copepods, protozoan, rotifers, cladocerans that aids to test the human impacts on ecosystem. Hence, this review article will present about main researcher working, foremost advances and discoveries in the field of utilization of Ostrocodes as bioindicators of environmental pollution.

2. ZOOPLANKTON AS BIOINDICATOR

Zooplanktonare very weak swimmers, non motile minute creatures which can drift in sea water column, freshwater bodies or ocean and can move long distance. They move towards sunlight zone in search of food abundantly and few are deep ocean dwellers. The freshwater zooplanktons comprise of

- Rotifers,
- Protozoa,
- Copepods
- Cladocerans,
- Ostracods.

Planktonic protozoans belongs to the class of ciliated unicellular organisms. Due to their very minute size, most of the species are not sampled. Heterotrophic nanoflagellates are more abundant than ciliates in fresh water body.

Rotifers are short lived among the planktons which is influenced by temperature, food and photoperiod. Dhanapathi reported that during favourable conditions there is a rapid increase in their numbers.

Cladocerans are most nutritive species among crustaceans for numerous members of fishes. It largely depends on bacterioplankton, algae and samller zooplankton for their feed. They are sensitive against pollutants sometimes sensitive to contaminants even at a lower concentration.

Copepods have the longest appendages which aids them to swim and possess the toughest exoskeleton compared to other zooplanktons. Copepods are of three oders, harpacticoid, cyclopoid and calanoid with different food habits. Cyclopoid are commonly carnivorous but feeds on bacteria, algae etc. Calanoid groups are generally omnivorous. Third group are primarily benthic where their variable feeding habits and physical structures helps them to live in harsher environmental conditions compared to others.

Ostracods are generally dwellers of benthic zone and they habitat on dead phytoplanktons and in sedimentation. These organisms are benthic macro invertebrate and food of fish.

To monitor the quality of marine ecosystems and freshwater these zooplanktons are used as bioindicators. Biotic indexes and bioindicators were used by the Europeans for about 100 years ago. These zooplanktons possess the property of bioindicators as their growth and distribution is based on some abiotic and biotic parameters. The abiotic parameters include temperature, salinity, dissolved oxygen, stratification, pollutants whereas the biotic includes predation, competition and food limitation.

Particular zooplankton's community status can indicate the trophic lake status . The lake water quality in Karnataka namely Fort lake Belgaum by analyzing the corresponding community size. As result of this survey, Rotifers recorded 52.38% of total zooplanktons, Copepoda 26.5%, Cladocerans 16.45% and finally Ostracods 4.67%. Thus zooplanktons play a huge role as bio-indicators.

3. OSTRACODES GENERAL INTRODUCTION

Ostracodes are the group of micro crustaceans which are known for their fossil records which belongs to the kingdom animalia and phylum arthropoda (Table 1). They are also known to be Mussel/Seed Shrimp as they resemble seeds. These are bivalved Arthropods enclosed within a low magnesium calcite that are easily fossilized and are present over 450 million years including evidences from the Oridivician. They are omnipresent and are wide habitats from tropical warm waters to polar very cold environments and were found from deep sea to intertidal zones. They are also adapted to freshwater niches such as rivers, lakes and even temporary ponds. Most species reproduce sexually but some of them reproduce asexually by parthenogenesis. The size ranges from 0.2 to 2.0 mm but the largest known species "*Gigantocypris sp.*" which measures upto 32 mm (Figs.1& 2) [1].

Table 1. Phylum Classification of Ostracodes

Kingdom	Animalia
Phylum	Arthropoda
Sub phylum	Crustacea
Super class	Oligostraca
Class	Ostracoda
Sub classes	1. Myodocopa 2. Palaeocopa 3. Platycopa 4. Podocopa 5. Metacopa

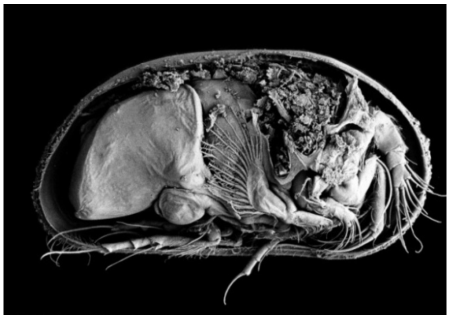

Fig. 1. Internal morphology of the Ostracods of *Cyprideistorosa* (male) [2]

Fig. 2. *Gigantocypris sp*. The largest known Ostracods species found

3.1 Morphology of Ostracodes

The Ostracodes are found with very shortened body and with bivalve shell made of chitin that are largely impregnated in most species with calcium carbonate. It includes 8 pair of appendages without growth lines. Head has four appendage pairs that assist in swimming, walking and feeding. Two pairs of thoracic appendages are useful in feeding, creeping, shell cleaning (Fig. 3).

It undergoes 8 stages of molt during the younger period. During the stages of molt, there is a change of shape, function and size of appendages, where these differences are much useful in deciding the instar collected, or whether juveniles as well as adults are present. Mostly they are benthic organisms and free- living because of their hard shells but some host in the phytoplanktons.

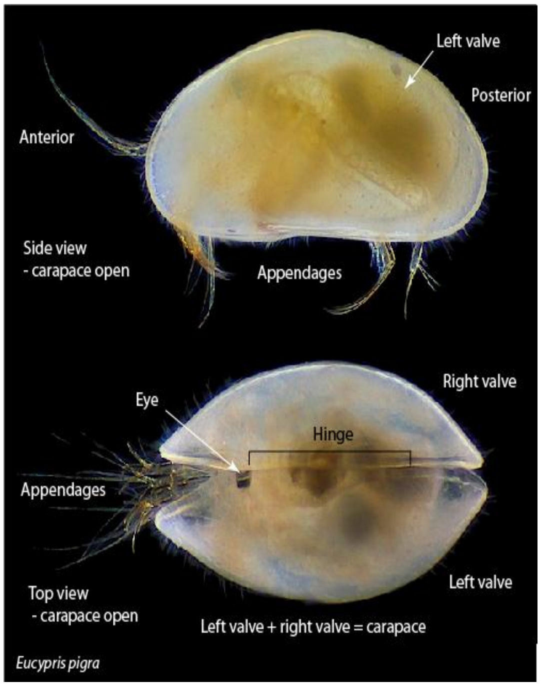

Fig. 3. Side view and top view of the ostracods (*Eucyprispigra*) with carapace open [3]

3.2 Characteristics of Ostracodes

3.2.1 Largest Sperm

Sperms of Ostracod Cypridoidea group are colossal and are often much longer (at least having one-third of their corresponding male length) [4]. The Australian species *Australocypris robusta* have the longest sperm, reaching length of 11.7 mm *i.e.* 3.6 times of the male length (Fig. 4).

Few of the insects have longer sperms, such as *Drosophila bifurca*the fruit fly, that can reach the sperms length of 58 mm. But ostracod have much thicker sperm, so the corresponding have much greater volumes; a single sperm volume is six times higher in the ostracod *Australocypris robusta* than in *Drosophila bifurca* (fruit fly). Additionally, while the sperms have long flagella in *Drosophila bifurca* but there is no flagella inostracod sperms. Every sperm has an nucleus that are extremely stretched out and wrapped up of two giant mitochondria for its lot of length .

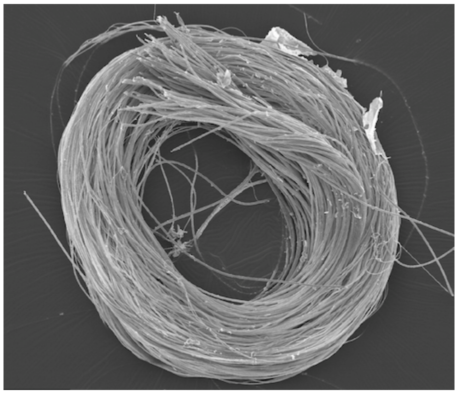

Fig.4. Giant sperms of *Australocyprisrobusta*.The male coils them into a ring to store the sperm [5]

3.2.2 Fossils record

The first ostracods fossil were found in Ordovician period from the rock. Due to their calcitic carapace, high fossilization potential is found in ostracods, and this combined with wide habitat preferences and huge diversity resulted it as most preserved arthropod in the fossil record. This makes ostracods much beneficial for palaeontologists, as they are used for palaeo-environmental (*i.e.* salinity, depth, temperature) and stratigraphical indicators.

Penis, the oldest fossil known belongs to the ostracod *Colymbosathon ecplecticos*, discovered in England (425 million years ago) [5]. An ash fall from a volcanic eruption had killed a numerous variety of animals that lived 425 million years ago in the sea. The fast decaying soft parts of the animals were preserved by this ash. A preserved painstaking 3-D reconstructions of male ostracod recorded wonderful features such as the gills, a penis and hairs on limbs.

3.2.3 Reproduction

Many ostracods that are living freshwater reproduce asexually by cloning themselves called parthenogenesis that are thought to be originated from a parasitic bacteria which affects the eggs and such species comprise the female populations. Species shus as *Dolerocyprisikeyai* exhibit geographic parthenogenesis, mostly with asexual populations and in a few isolated places with sexual populations.

In asexual reproduction, there are few short term advantages *i.e.,* there is no watste of energy or time in searching a mate or producing males and only a individual is required to start up a new population, hence these species can spread faster than their sexual counterparts. Moreover, long term reproduction without sex creates genetic mutations to build up and asexual populations are condemned to extinction.

3.2.4 Eggs

Temporary water bodies (puddles and rice fields) are found with many freshwater ostracods. The survival in such temporary habitats is because their eggs are viable even when they are dried. The eggs develops and hatch when the water is available.

A famous Norwegian G.O. Sars, a crustacean worker, used this ability for the ostracods study from the other side of the globe. In Australia and South Africa peoples sent him their collected dried sediment from their rivers or ponds that are dried to him. The dried sediment are added with water and after few weeks, G.O. Sars raised the ostracods and found that many of them were new species, that were then named and described.

3.2.5 Bioluminescence

Some Myodocopida ostracods species produce a blue bright light. In the world places such as Australia, Japan and the Carribean, at night are recorded with light flashes in water. This light produced is done with the presence of O_2 by mixing of two chemicals. Two enzymes involved are luceferin and luciferase. Luciferin reacts to produce light with oxygen, while luciferase (enzyme) as a catalyzer speeds-up the process. To stop confusions in dark, the species flashes at different rates.

The luminous systems used by a number of species are not rigorously endogenous and either the whole luminescent system or the substrate of the photogenic oxidation could be of exogenous origin. In the latter case, the lueiferins appear to be imidazolopyrazines derived from either eoelenterazine, or from luciferin of the ostracod, *Vargula*. Fig. 5 shows the bioluminescence property of *Vargula sp.*

Fig. 5. A female *Vargula sp.* (2.4 mm length) exhibiting bioluminescence property [6]

4. APPLICATIONS OF OSTRACODS

- Palaeoenvironmental
- Bio indicators
- Palaeoclimatic reconstruction
- Palaeobathymetry
- Biostratigraphic indicator
- Ecotoxicity monitoring
- Palaeosalinity

4.1 Paleoenvironmental Application

Ostracods have a long history of provision to geology. Towards the palaeoecological reconstructions Charles Lyell relised the worth since 1824 in Scottish Quaternary lake deposit, incase of Edward Forbes used during 1851 for subdividing the Upper Jurassic Purbeck Beds in southern England with possibly the oldest zone proposal based upon microfossils. Ostracods are possibly best known for this, where their abundance through a wide diversity of aqueous habitation allows a unique involvement from micropalaeontology to the understanding of primordial environments [7].

4.2 Bio Indicators

The ostracods in Freshwater habitat are more sensitive to environmental conditions in their territory. In the aquatic environment ostracods act as biological indicators since the Palaeozoic eraand therefore involved in the whole fossil record of any present arthropod group [8]. Rice biodiversity and well conserved in lacustrine sediments, ostracod valves offer an outstanding microfossil record [9,10]. Therefore, freshwater ostracods are of great interest as biological indicators of environment and changes in climate of the Quaternary past and in modern studies [10-12]. Freshwater ostracods obtained from permafrost areas are valuable biological indicators of aquatic environment since permafrost deposits have a high conservation potential, fossil ostracod assemblies provide comprehensive perceptions into past environmental situations, if current reference data sets of limnological data and species archives are available for evaluation. This obligation highlights the requisite for additional organized studies of present environmental underlying forces and ostracod assemblages [13]. The first discovery of the species *Fabaeformiscandona groenlandica*, *F. krochini* and *Fabaeformiscandona* sp. was done in the Indigirka Lowland in north east Siberia [13].

4.3 Paleoclimatic Reconstruction

Underpaleolimnology ostracods are recently identified as important group of organism and as an essential mechanism for research concerning climatic change. With the rising facts of temperature and oxygen stress of species, their use in the study of thin lakes with respect to climate and lake level changes and the onset of meromictic conditions has become more and more important [14].

4.4 Paleobathymetry Application

Concerning the identification of paleoenvironments, ostracods are a significant constituent of marine sediments. They provide a novel and hopeful tool for future studies of paleobathymetry based on ostracod distribution at continental margins. The results of this study and additional study on two other transects from continental margin could be applied as a model to reconstruct the paleoenvironments in Indonesia [15].

4.5 Biostratigraphy

The previous studies on biostratigraphic were based upon the planktonic foraminifera, palynomorphs and nanno fossils that are very poorly constrained in age and had impeded the correlation with those of other units belonging to the Atlantic Equatorial margin. During Neogene, the tectonic stability of the Northeastern Amazonia coast were associated with the continuous rise in sea level and decreased riverine inflow, allowing the establishment of the tidal channel, mangroves/lagoon and the shallow platform settings that favors the massive proliferation of the benthic ostracods. The repetitiveness of these system of deposition along the whole Oligocene-Miocene succession had contributed mainly to preserve the typical assemblages of the lagoonal ostracods. Among the 32 identified ostracod genus, most of them belongs to Polyhalines that were associated with a mesohaline genus as Perissocytheridea. Rare genera of ostracods that are typical of the offshore zone indicated their limited oceanic connection with the lagoon. In addition, the presence of Cyprideis rarely, shows a relatively stable degree of salinity suggesting the change in lagoon dynamic until the estuarine conditions. There are more than 100 ostracod species ranging from the upper oligocene to the lower Miocene with a five index species that corresponds to a single zone that are called as zone of *Cytherella stainforthi* that were further subdivided into four sub-biozones *viz., Quadracythere brachypygaia*, *Jugosocythereis pannosa, Neocaudites macertus* and *Triebelina crumena*. The assemblages of ostracod that were described here provides a classic biostragraphic framework for the local, intrabasinal and also for regional correlation with Oligocene-Miocene deposits of the Caribbean regions and Northeastern Amazonia coast [16].

4.6 Ecotoxicity Monitoring

Othman et al. described as the adults of freshwater ostracod *Stenocypris major* (Candonidae, Crustacea) were exposed in laboratory conditions for a four-day period to a range of cadmium (Cd), copper (Cu), zinc (Zn), copper (Cu), nickel (Ni), copper (Cu), aluminium (Al), manganese (Mn) and iron (Fe) concentrations. The mortality was recorded, and the median lethal times (LT50) and the concentrations (LC50) were calculated. It was found that the LT50 and LC50 increased when there is a decrease in the mean exposure concentrations and times, respectively, for all metals. LC50s for 96 hours for Cu, Cd, Pb, Ni, Zn, Fe, Mn and Al were 25.2, 13.1, 526.2, 19743.7,1189.8, 278.9, 510.2 and 3101.9 µg/L, respectively. The bioconcentration of metals in *S. major* increased with the exposure to the increasing concentrations, and Cd was found to be the most toxic to *S. major,* that werefollowed by Cu, Fe, Mn, Pb, Zn, Al, and Ni (Cd > Cu > Fe > Mn > Pb > Zn > Al > Ni). The comparison of LC50 values for the metals for *S. major* with those for the other freshwater crustacean showed that *S. major* is equally or highly sensitive to the metals than most of the other tested crustacean. The above study indicated that *S. major* is the potential bioindicator organism for metals pollution and in the toxicity testing [17].

4.7 Paleosalinity

Ostracods are the common most lacustrine calcitic microfossils. Their faunal assemblage and the morphological characteristics are vital ecological proxies, and their valves are the archives of geochemical information that are related to palaeohydrological and palaeoclimatic changes. In the order to assess ostracod ecology (Valve morphology and taxonomic diversity) that were combined with valve geochemistry ($\delta^{13}C$ and $\delta^{18}O$) as the palaeosalinity indicators, we analysed the sedimentary material from the ICDP (International Continental Scientific Drilling Program) Ahlat Ridge site from a alkaline and terminal lake, Lake Van in Turkey, covering upto the last 150 kyr. Though the diversity of the species are low, the faunal assemblage of ostracod reacted sensitively to the changes in concentration of the total dissolved salts in their aquatic environment. *Limnocythere inopinata* is found throughout the studied interval, while *Limnocythere* sp. A is restricted to the Last Glacial period and are related to the increased lake water alkalinity and salinity. The presence of the species belonging to genus *Candona* are limited to the periods of lower salinity.The valves of the Limnocytherinae species (incl. *L. inopinata*) display the nodes (hollow protrusions) during intervals of higher salinity. Both the number of noded valves and number of nodes per valve appeared to be increasing with rising salinity, suggesting that formation of node is related to that of the hydrological changes (alkalinity and/or salinity). In contrast to the Lake Van's bulk $\delta^{18}O$ record, $\delta^{18}O$ values of the ostracod valves do record a relative changes of the lake volume, with lesser values during the high lake level periods. The $\delta^{13}C$ values of the different species reflects the preference of ostracod habitat (*i.e.* epifaunal vs. infaunal) but were less sensitive to the hydrological changes. However, when combined with other proxies, the decrease in Holocene $\delta^{13}C$ values would indicate the freshening of the lake water compared to that of the low lake level during the Last Glacial period. The Lake Van example underscores the significance and value of coupling ostracod ecology and valve geochemistry in palaeoenvironmental studies of the endorheic lake basins[18].

5. OSTRACODS AND ENVIRONMENTAL PARAMETERS

Either the development of an ostracod assemblage or a single species is influenced by physio-chemical water properties of such as temperature, salinity, dissolved oxygen, pH, hydraulic conditions, sedimentation rates or bottom grain sizes [2]. In addition to community changes and population, geochemical and morphological alternationare recorded in ostracod carapace, that serves as a water quality tracer.

All these features allow to set the spatial outcome of mining effluents, urban sewages, road building, watershed deforestation or agricultural wastes. These data forms the basis of the reconstruction of cores in palaeo-environmental, with archaeology application.

5.1 Physical-Chemical properties

5.1.1 Salinity

Salinity (concentration of total major ions that are expressed in mg/L) and solute composition (the major ion's water compositio expressed typically in mg/L or meq./L) are needed to biogeography ostracod physiology and ecology. In North America, range of natural water salinities are found between extremely dilute (5 mg/L recorded in NANODe) to the hypersaline conditions (>400 g/L). Non-marine ostracods occur in the lower range (5 mg/L to 100 g/L), although many species are restricted to less than 30 g/L. Osmotic regulation is physiologically disturbed by salinity and hence balance between regulation and calcificationion. Some species of euryhaline are found to be present in limnic waters but can also inhabit even in environments that are hypersaline, whereas others such as *Candona candida, Ilyocypris bradyii, Herpeto cyprisreptans, Fabaeformis candonalevanderi and F. protzi,* are restricted to 6% salinities (Fig. 6).

In dilute water below 300 mg/L, the major ions found are bicarbonate, magnesium and calcium. With increasing salinity, concentrations of ions increases, until calcite saturated and the precipitates at approximately 300 mg/L(total ionic concentration). These are said to be the calcite branch point (first mineral branch point) that occurs in natural waters. After which, the water depletes for calcium and gets enriched for bicarbonate, or *vice versa*, out-turns in a solute path that are calcium-depleted, bicarbonate-enriched saline water or calcium-enriched, bicarbonate-depleted saline water.

Eventually, other mineral branch points such as gypsum, would be reached and some ion composition changes occurs. Hydrochemically, the water body fate in an evaporative setting is decided at the calcite branch point and some solute path changes occur by addition of water that is hydrochemically different.

Rather than salinity, determination of species present becomes difficult when the water ion composition is beyond the calcite branch point. For example, different *Limnocythere* species in lakes are found with the same salinity but of different composition of ion [19]. There are boundaries, identified by Frorester as *hydrochemical ecotones*, that defines the species habitats (Fig. 7). Although the genus *Limnocythere* is an example for partitioning of hydrochemical, most of the other species of ostracode response to these differences in solute. Thus, within lakes, the reconstruction of changes in hydrochemical is done with the knowledge on response of species to different solute paths.

5.1.2 Temperature

Water temperaure of the aquatic habitat plays a important role in the geographical habitat and in distribution of seasonal ostracodes. Other factors such as solute composition, availability of food, energy levels and turbidity are also considered. The temperatures range from 0°C to over 30°C for shallow water habitats depending upon altitude and latitude. Species that are living in this niche must have tolerance ability for these broad ranges in one or more stages of life. Species such as *Candonaacutula*, *C. ohioensis*, *C. candida*, *Cyclocyprissharpei*, *C. ampla*, *Limnocytheres taplini* and *Cypria ophthalmica*, displays a range of bottom water temperature. The exceptions to the above all are the shallow groundwater-fed streams that might have nearly constant temperature.

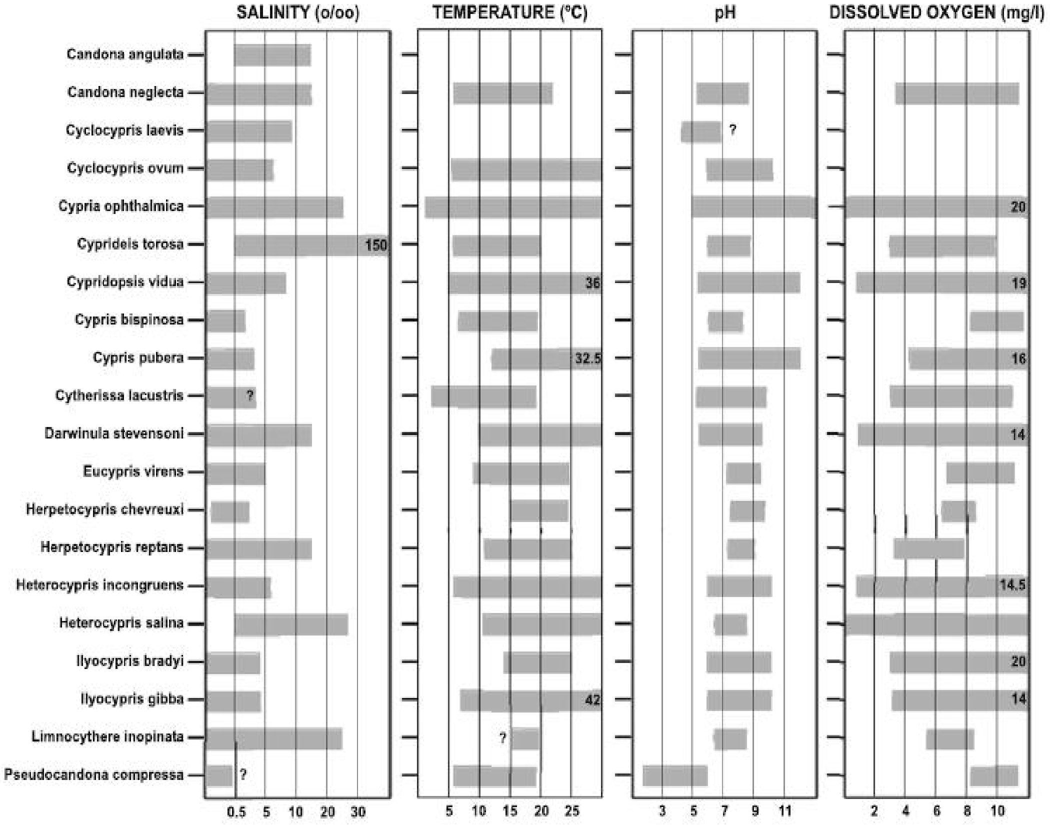

Fig. 6. Physical and chemical conditions of 20 selected freshwater species [2]

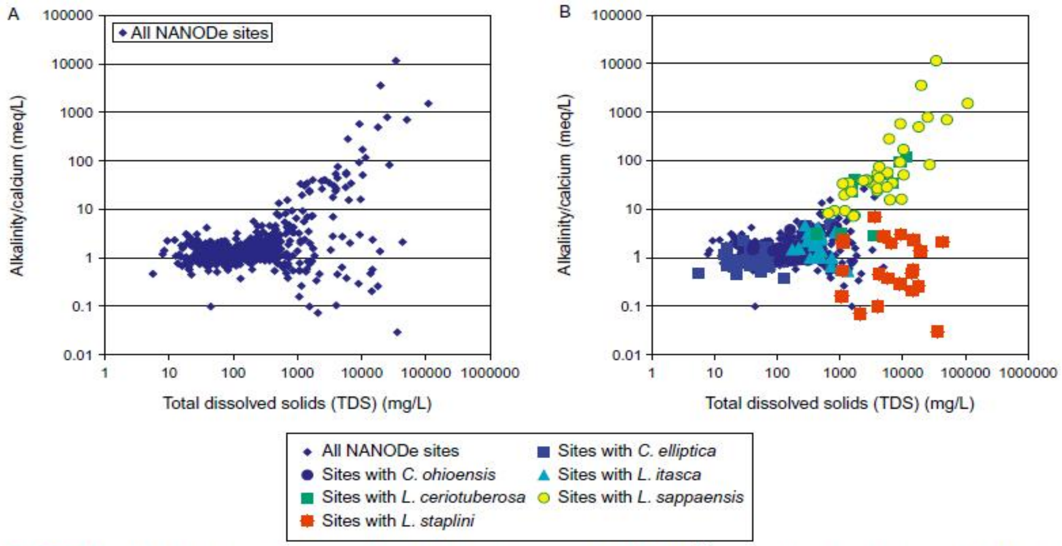

Fig. 7. Major ion hydrochemistry of nearly 600 surface water sites in NANODe showing: A - Distribution of solute alkalinity/calcium (meq/L) versus rising concentration as total dissolved solids (mg/L) and B - Selected ostracode species distribution exhibiting preferences for ranges of ionic concentration and composition [2]

Candona subtriangulata has a range of 2.6°C–19.2°C with low mean value (5.5°C). This species are commonly present in Lakes Ontario, Superior and Huron at considerable depth, where there is no much difference in bottom water temperature. Several experiments are done with *Cypriaophtalmica* and *Cypridopsis vidua*, in which for several hours they were frozen in ice . This ice was then melted and found that most of the specimens survived after freezing experiment.

At the other extreme of temperature, *Cypris balnearia* has been described from thermal springs (45°C-50.5°C). Some researchers in Oregon, recovered *Potamocypris* from an algal–bacterial substratum of a hot spring (30°C - 54°C). *Chlamydothe caarcuata* ound in warm springs (Nevada, Utah, Mexico and Arizona) where the temperature of water varies between 24°C and 39°C and In Nevada, *Thermopsis thermophila* recorded from a hotspring of 4°C – 50°C .

5.1.3 pH

Ostracods in freshwater prefer slightly acidic or alkaline waters, although few species can adapt a wide pH range (4.6 to 13) (Fig. 6) and others were seen even in high acidic waters. In general, species of ostracod are not seen at a pH greater that five, as in acid waters uptake of calcium is difficult for carapace calcification [20,21].

5.1.4 Dissolved oxygen

Dissolved oxygen is needed for survival of aquatic habitat. The mean requisite of ostrocods falls within a narrow margin of 7.3 mg/L– 9.5 mg/L. Generally, the dissolved oxygen concentration of water is broad. The *Candona subtriangulata* requires low oxygen (5.6 mg/L). A number of species are found that can tolerate low concentrations of dissolved oxygen (Fig. 8).

The species such as *Cytheromorphafuscata*, *Cytheris Sa lacustris*, *Potamocypris variegata, Limnocythere ceriotuberosa*, *L. verrucosa*, *L. itasca*, *L. herricki* and *Ilyocypris gibba* requires a minimum dissolved oxygen of 3 mg/L. *Fabaeformis candona caudata* can survive even if the hypolimnion has summer dissolved oxygen falls below its lower tolerance limit (2.8 mg/L) in Lake Erie [22]. Before onset of anoxia, its survival was by allowing it for egg production by having short life cycle. In Lake Erie, these types of survival mechanism are seen for some time as indicated by the existence of lake sediments with fossil shells. Rather in Lake Erie, *F. caudata*, *Cytheris Sa lacustriv and C. subtriangulata* have become extinct locally because of its one year life cycle and couldn't reach sexual maturity as they require a minimum content of dissolved oxygen. *Cytheris Sa lacustris* comes back with anoxia reduction.

5.1.5 Nutrient levels

Increase in concentration of several pollutants effects number of ostracode species. Herpetocypris gets disturbed by high amount of phosphate, whereas *Candona neglecta* are remarkably affeted by higher nitrate content and Some species such as *Ilyocypris inermis* are absent in high nutrient level disturbed sites [23].

5.1.6 Depth water

Although it is much hard to get a statistical correlation between either individual species abundance or ostracod diversity and depth, some common patterns are laid in stable environments of freshwater (Fig. 9). Some species *(Ilyocypris echinata, Darwinula stevensoni, Limnocythere inopinata*) are typical of the benthos of shallow areas, whereas *C. candida, Candona angulata, Cypria ophthalmica, Crypto candona reducta, Cyclocypris ovum, Limnocythere sanctipatricii, Potamocypris smaragdina or Cytheris salacustris,* can also be found in deeper benthos that is greater than 40 m in depth [24].

Climatic changes relates to the water depth in some areas. In temporal lakes, during wet and deep-water periods, species that can swim are dominant, whereas burrowing species are seen in dry, shallow-water periods and few individuals are only found when the lakes dry out completely [25].

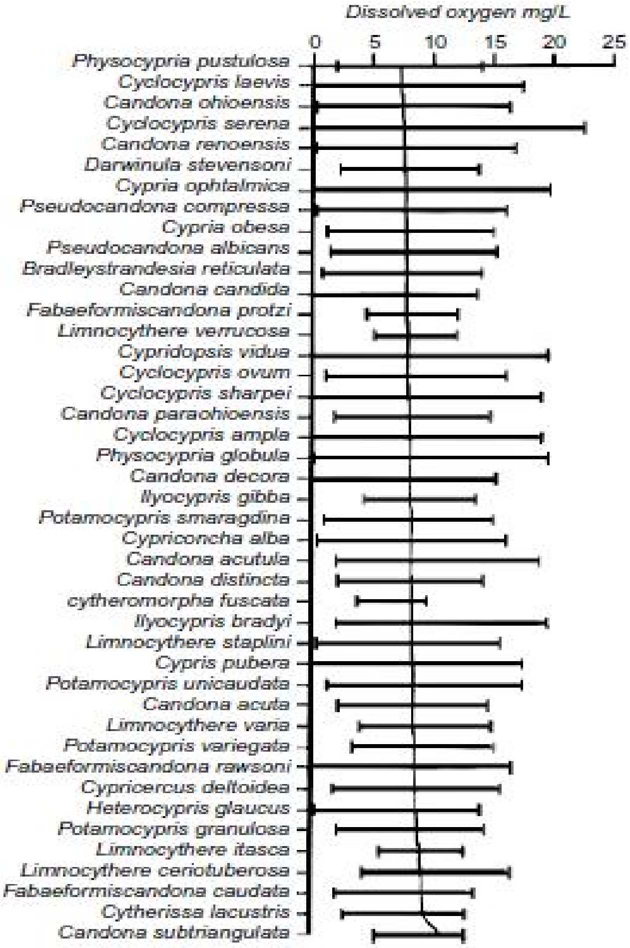

Fig. 8. Dissolved oxygen range for some Canadian freshwater ostracodes. The mean represented by solid line and the minium and maxium values represented by bars [22]

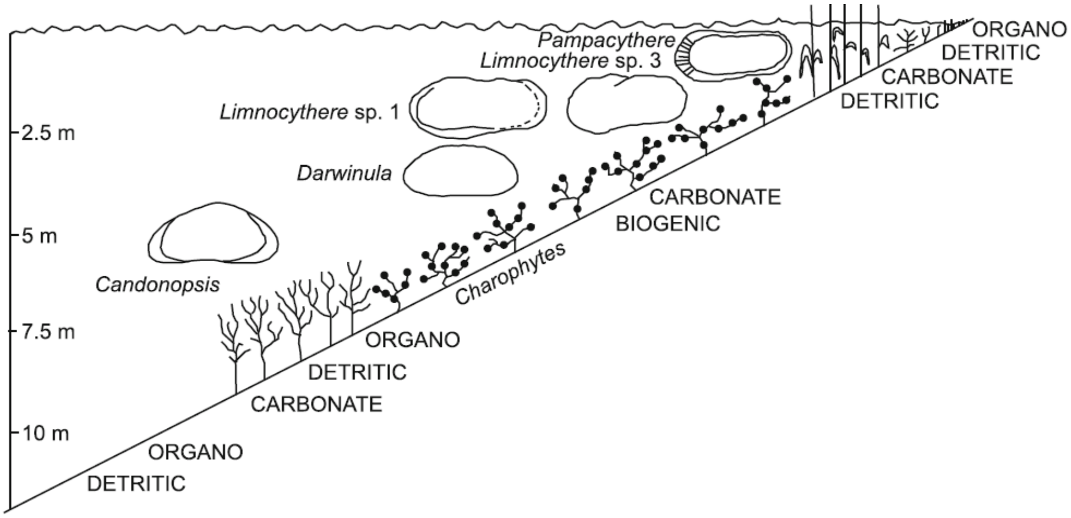

Fig. 9. Distribution of the important groups ostracods species related to sediment type and water depth in Lake Huinaymarca at Bolivia [2]

5.1.7 Hydraulic conditions

Increased in water velocities is avoided by ostracods by going inside vegetation or sediments, athough they may be found fast flowing streams. Additionally some of the species are present with positive significant correlation to water turbidity [26]. Moreover, the distribution of thanatocoenosis may be seasonal indicative of hydrodynamic conditions. Isolated valves species of freshwater such as *Lineocypris sp.* and *Cytheris salacustris* during high flows are transported even upto 2 km seaward, whereas in the same site during low flow only few marine species are found.

5.1.8 Species traits and habitat utilization

Late maturity, long life spans, geometric carapace shape, low migratory ability, low fecundity and medium size are characters of ostracods species living in hypogeanand interstitial. Standing surface waters and in permanent flowing waters, the epigean species that are abundant have various body forms, large size, long life spans and some give parental care. Rather, in stagnant waters and temporal ponds, many species have high migratory ability, high tolerance desiccation, short life spans and have cylindrical or spherical shape. The overview of ostracods carapace make us to understand about the environment stability/instability. Candoninae species have trapezoidal, elongate or triangular valves with precise posterior margin in environments that are, whereas unstable environments are characterized by sub-circular outline in Cypria species [27].

5.1.9 Grain size

The grain size influence is variable on assemblages of ostracods. In some lakes, survival of ostracod falls off with particle size reduction [28] whereas the effect is insignificant on ostracod populations grain size distribution of river biofacies and some freshwater ponds.

Some species including *L. sanctipatricii, Limnocythere inopinata, Ilyocyprisbradyii and Leucocythere mirabilis,* usually occurs in fine-grained sediments, contrarily with the high microcrustaceans abundance that were found in coarse facies of some alpine streams and karstic areas. Moreover, the salinity and parameter changes might show remarkable effect of selected species on the patter of ornamentation in other areas.

5.2 Ostracod Carapace as Tracer

5.2.1 Ornamentation

Ornamentation of external surface are applied in several environmental studies. *Cyprideistorosa, Cytheris salacustris or Limnocythere inopinata* exhibited punctuated, smooth, noded or reticulated carapaces (Figs. 10 & 11) depending on range of salinity [29] and freshwater environments are found with rounded pores, whereas oligohaline to hypersaline waters have irregular pores. *C. torosa*noding problem is mostly an controlled osmotically one, as noded specimens are not only present in low salinity waters but are also with low calcium content [30]. The ecophenotypism in species of ostracod might be due to multifactorial system. The temperature influence cannot be restricted. The most reticulated carapaces are found to be with rich source of magnesium.

6. GEOCHEMISTRY

Useful information on palaeoenvironmental can be provided by fossil carapaces's stable isotope geochemistry and trace elements [32]. In a freshwater species, carapace phosphorus content (% or ppm) might be identical in the same environment and can alter between various geographical localities, that are the indicative for changing geochemical conditions. Besides, it is necessary to indicate analysis position in the ostracod carapace, as there is change in elemental percentages between external or internal zones of the similar carapace. If the elements related with mineral inclusions have a rather homogeneous distribution, those entail in biological pathway (Na, P, Ca, Mg, S) are in heterogeneous distribution.

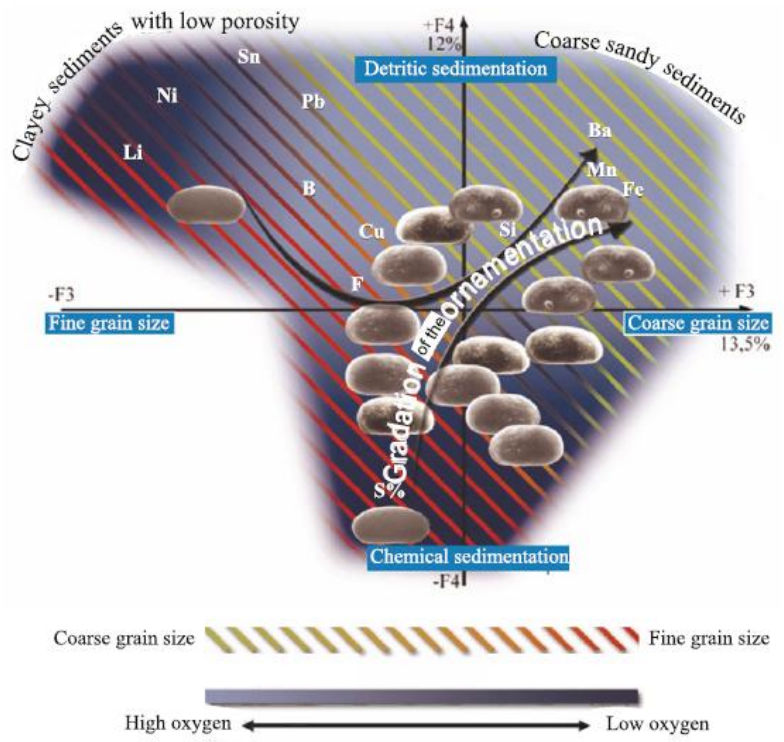

Fig. 10. Influence of granulometry (edaphic support) on the ornamentation of *Cyprideistorosa* [31]

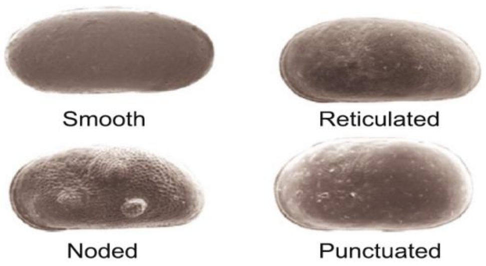

Fig. 11. Various types in ecophenotypic ornamentation of *Cyprideistorosa* [2]

7. MINING/INDUSTRIAL WASTES AND URBAN EFFLUENTS

Wastes obtain from various pollution sources causes relevant changes on density and diversity of ostracods. In highly organic-polluted waters that are near to industries or urban, ostracods are scarce usually and can disappear even [33]. These effects declines usually downstream in some of the rivers, with the appearance of various ostracod assemblages through a gradient from the high pollution to the ending zone of ‘‘recovery’’ (Fig.12). Similar effects are produced by mining activities on ostracod assemblages, with a fast decrease in species that are found near the treated polluted underground waters. some ostracod species (*Herpetocypris chevreuxi)* can eliminate a part of the pollution [2].

8. HERBICIDES AND PESTICIDES

Various experiments had analyzed the outcome of variable pesticides or fertilizers doses on various species of ostracod (Fig.13) [34]. These short studies, in most cases on 24–96 hours of exposure time to toxins, showed that these microcrustaceans were very good bioindicators, with a sensibility similar or higher to amphipods, copepods, cladocerans, prawns or crayfishes (Australian and New Zealand Environmental and Conservation Council 2000).

Low doses of pesticides (mexacarbate or DDT) or herbicides (dioxin) results in initial accumulation on the soft parts, also rising the other pesticides concentrations provoke immobilisation , intoxication, or even the mortality in species populations of *Cypretta spp., Heterocypris incongruens, Cypridopsis spp. or Eucypris sp.*(Table 2).

9. AGRICULTURAL WASTES

The massive and widespread use of herbicides, pesticides and fertilizers lowers the richness of ostracod [35], although few species are resistant to organic pollution or pesticides. Tolerant taxa in lowland springs are dominant with high content of nitric nitrogen (>800 μM) derived from a diffuse pollution originated from agriculture [36], similarly rise of phosphate contents are connected with the absence of living specimens in some upper lakes sediments [37].

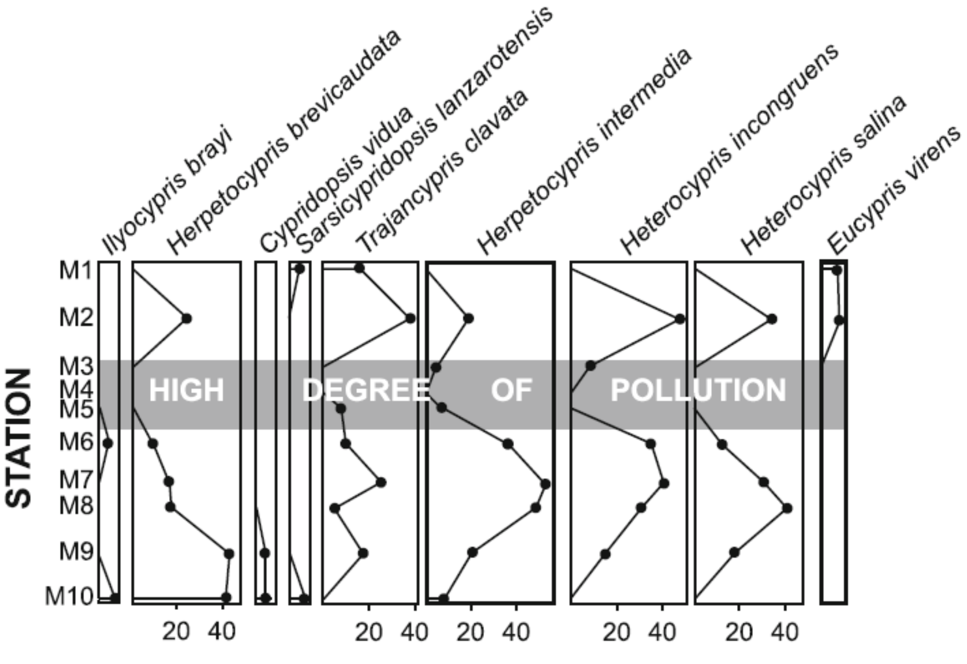

Fig. 12. Impact of ostracod populations due to industrial effluents of Magre River (Eastern Spain), with transformed average density values calculated form sampling taken from seven month field [2]

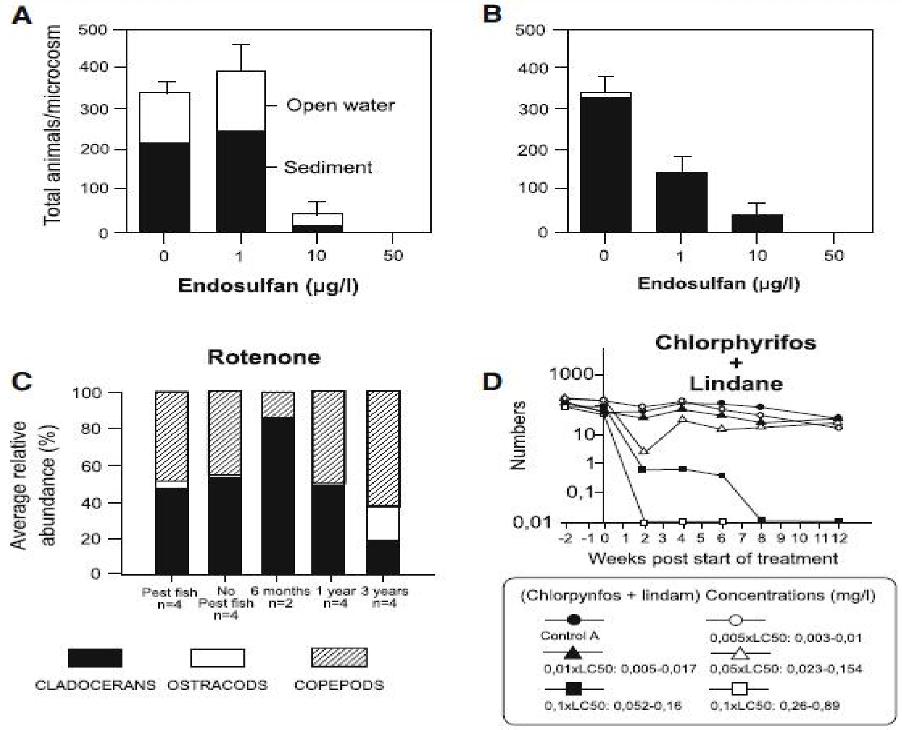

Fig. 13. Impact of the Endosulfan - organochlorine pesticide (a, b), Rotenone - the broadspectrum insecticide (c) and Chlorphyrifos and Lindane (d) on ostracod populations. a, b - Effect of endosulfan on total numbers ± stand error of ostracods in microcosms of *Cyprettasp* and *Eucypris sp*. 10 weeks after initial application in Australia and southwestern Victoria (modified from Barry and Logan 1998). c -Mean relative abundances (%) of zooplankton collected in sweep-nets in 18 orchard ponds at 5 treatment levels in the Motueka region, New Zealand (modified from Blakely *et al.* 2005). d - Changes in number of ostracod taxa by treatment level of Ostracoda expressed as the geometric means of counted numbers (modified from 34)

Table 2. Effects of pesticides (P) and herbicides (H) on Ostracod populations

Name	Formula	Type	Ostracod species	Time study(h)	Toxic dose (µg/l)	Effect	Source
Lindane	$C_6H_6Cl_6$	P	*Heterocypris incongruens*	24	2.07-6.9	Accumulation	[38]
Molinate	$C_9H_{17}NOS$	H	Not detected	48-96	180-33,200	Ostrcodes and cladocerans were more sensitive than crayfishes and prawns	[39]
Trifluralin	$C_{13}H_{16}F_3N_3O_4$	H	Not detected	48-96	37- 2,200	Immobilisation	[40]
Cadmium chloride	$CdCl_2$	P	Cypridopsis sp.	96	190	Mortality	[41]
Endrin	$C_{12}H_8Cl_6O$	P	Not detected	48-96	0.5-74	Ostracods and prawns as the most sensitive groups	[39]

10. PALEOENVIRONMETNAL APPLICATIONS

Various studies are focused based on a multivariate analysis (mineralogical data, stratigraphic units, isotopic trends, macro and microfaunal assemblages) on palaeo-environmental reconstructions of cores collected in freshwater environments, along with the analysis of ostracod [42]. Auto ecological stratigraphic analysis of ostracod assemblages in lakes is especially interesting where the perseverance of local populations is regularly threatened by changes and disturbance in both water quality and availability. The studies carried out on sediment cores, population age structure or in ostracod assemblage change are related to the new predators introduction, freshwater drainage channel construction, or rise of agriculture water management. Contrarily, alternatives of marine/brackish or freshwater associations let the recognition of variations in salinity that could be associated to palaeogeographical reconstructions or Pleistocene sea level changes, salinity and lake-level variations [43,44].

These assemblage changes, along with isotopic studies applied to ostracod carapaces, are useful in reconstruction of depth-water variations, climatic changes (cold/warm phases) or hydro chemical/hydrological conditions [45]. Some ostracod species morphological features (shape, size) were used in the reconstruction of palaeoenvironmental conditions. Prevalence of geometric and large sized ostracod carapaces stipulate a stable environment and the existence of various morphologies allows to attest rift activities, without any sedimentology variations.

Nevertheless, Holmes [46] showed numerous problems related to:

- Methods used for ostracod shells extractions and their subsequent cleaning from sediments.
- post-mortem shell alteration and diagenesis
- Obstacle with calcification mechanism
- Temporal and spatial variability in composition of shell
- Individual specie's ecological tolerances
- Relationships between palaeohydrology and shell chemistry.

10.1 Aral Sea Ostracoda as Environmental Indicators

Variations in the level and interaction during its history have played a key part in determining the floral and faunal populations of Aral Sea. Out of the eleven species studies, Ostracoda (Crustacea) known to have been existing in the Aral Sea since 1960, only one lives today due to the anthropogenically induced salinity rise of the past three eras. Origins of mixed fresh and brackish water ostracod are deliberated, and it is concluded that some of the major fauna must have reached the Aral Sea during a past in elevated water level phase when assembly existed through the Caspian Sea [47].

11. PALAEOCLIMATIC RECONSTRUCTIONS

Lacustrine ostracods are used as palaeoclimatic tracer. Isotopic analyses (^{13}C and ^{18}O) of ostracod carapaces aids to have a clear idea on regional events [48] or feasible exchanges between seas and lakes [49]. These studies, further in conjunction used with protein dating by amino-acid racemization to give useful details about palaeotemperatures.

12. ENVIRONMENTAL ARCHAEOLOGY

These previous applications were much helpful in archaeology, with a usual ostracods collection with fragments of foraminifera, plant remains, bones, molluscs, pollen and/or spores [50,51]. Griffiths et al. [52] details a useful summary of preparation, identification and sampling techniques.

The isotopic composition and shell chemistry can be used to reconstruct the climatic alternation related to various periods of old cultures [53]. The end of activity in old harbor channels could be inferred from the variations from marine to freshwater ostracod assemblages [54], whereas some of the ratios such as Sr/Ca and Mg/Ca have been used for understanding the prehistoric civilization's evolution (Fig. 7: Hohokam culture, Arizona; Palacios Fest, 1997).

13. HEAVY METALS

A ''culture/maintenance-free'' microbiotest has used the freshwater species *Heterocypris incongruens* (Table 2), specifying that the mortality of ostracod in Zinc polluted soils was due to the (non-soluble) toxicants that are bound to solid-phase particles, rather than those dissolved in water phase [55]. In recent years this test is used as part of a battery of bioassays to characterize the to fluvial sediments toxicity [56].

An additional bioassay test applied to *Cypris subglobosa* to measure the toxicity of 36 metals and 12 reference toxicants recorded that osmium was the toxic most in the test while least was boron. Rise in mortality of this species population was due to increase in water acidity and copper concentration. In other studies relating to toxicological this metal and cadmium were added between the most toxical to *Stenocypris* major [17].

In these waters that have been polluted, there is a survival of some freshwater species such as *Chrissiahalyi,* being the excretion of survival tolerance mechanisms. This mechanism efficiency knock down due to nominal amount of lead in water rises. Similarly, it is required to analyse the connection between the metal contents of both sediments and waters and diversity and abundance of ostracod [57].

14. OIL CONTAMINATION

Test of oil contaminated sediments in the experimental plot after 6 and 21 weeks, expressed that *Heterocypris incongruens* are highly sensitive than amphipod *Hyalellaazteca.* More recently, other species such as *Cyprettaseurati, Stenocypris hislopietc* were used for acute lethality test of biodegradable lubricants [58]. The longevity of *C. seurati*, that were active physically, was greatly affected by water pollution with tremendous effects, if copper containing oleic acid is used.

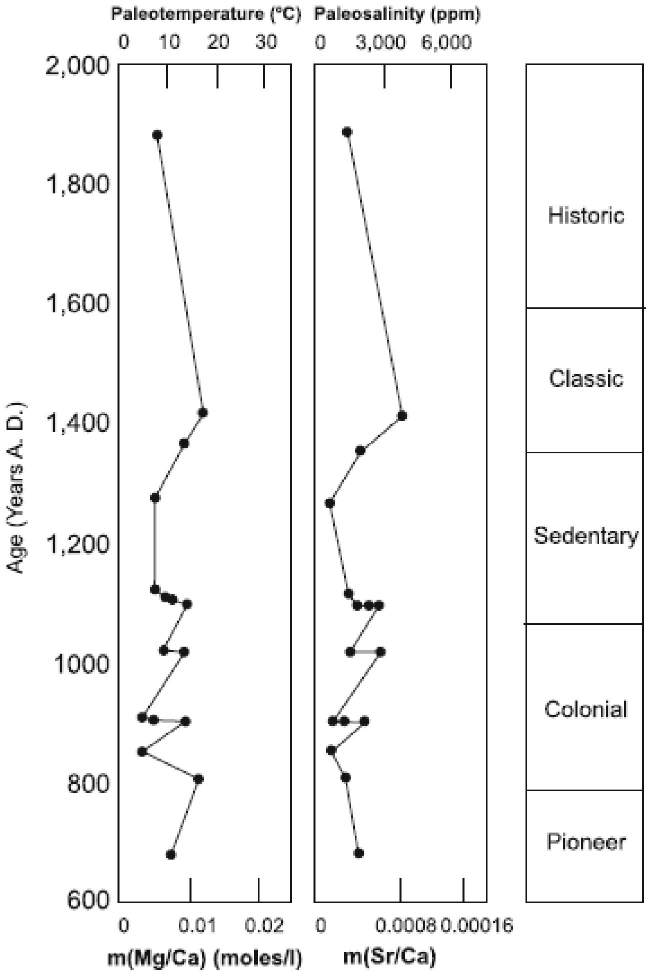

Fig. 14. Trace elements palaeoenvironmental reconstruction of Hohokam canals history (Arizona) of *Limnocytherestaplini*based on ostracod shell chemistry (modified from 57)

Table 3. Difference in the Ostracodtoxkit^{TM} microbiotest under various experimentally induced pollution with further well known microbiotests

Sl. No	Assessment	Contrast	Impacts	Source
1	Hatching time, mount of supplemental algal food, volume of sediment and period of the trial	Solidphase test of *Hyalellaazteca*	Test protocol for a 6-day assay in 12-cup multiwell plates with ten organisms per cup and three replicates. Calibrated sand as reference sediment. Mortality and growth of the ostracods determined after 6 days incubation at 25^{o}C in darkness	[59]
2	Oil contamination	95% Statistical confidence	Development of novel procedures: selection of a validity threshold for quantity of substrate (300 µL), number of replicates (6), mortality (20 %) and health of the trial organisms (600 µm)	[60]
		Hyalellaazteca solidphase test	Six weeks: Higher transience of Ostracods. Fifteen weeks: sediments still poisonous to ostracods but not to *Hyalella.* Lower variation coefficients amid replicas of the ostracod effects	[61]
3	‘‘Culture/maintenance-free’’ direct contact	*Hyalellaazteca*, *Thamnocephalus platyurus* and *Raphidocelis subcapitata*	Harmonizing information delivered by the four tests	[62]
4	Toxicity Assessment	Springtail *Folsomia candida*	Ostracod test species are more sensitive than the springtails.	[55]

15. CONCLUSION

From all the above applications it was clear that Ostracods a unique meiofaunal species is utilized in ecosystem in many aspects. The main advantage over this species is that it can be easily fossilized and it had escaped 5 extinctions which have the records from the Ordovician period. As it is sensitive to the physic-chemical properties such as pH, temperature, salinity, dissolved oxygen, sedimentation ratios of their habitat they are being applied for biostratigraphic studies, paleoenvironmental reconstructuion, paleo oceanography and paleo climatology etc. Not only have the physic chemical properties also the morphology and orientation of the carapace is involved in the deciding factored of community size (abundance and diversity).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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