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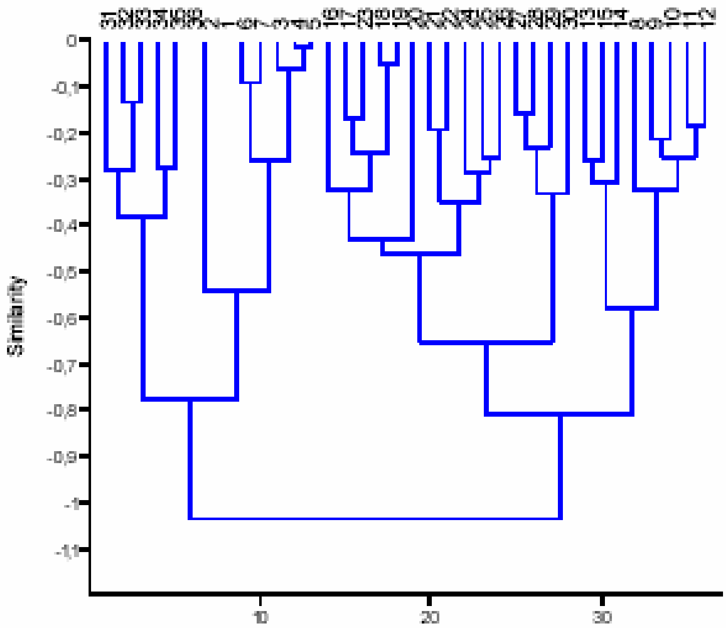

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A REVIEW ON PHYTOREMEDIATION OF HEAVY METALS AND UTILIZATION OF ITS BYPRODUCTS

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Abstract. This review presents the status of phytoremediation technologies with particular emphasis on phytoextraction of soil heavy metal contamination. Unlike organic compounds, metals cannot be degraded, and cleanup usually requires their removal. Most of the conventional remedial technologies are expensive and inhibit the soil fertility; this subsequently causes negative impacts on the ecosystem. Phytoremediation is a cost effective, environmental friendly, aesthetically pleasing approach most suitable for developing countries. Despite this potential, phytoremediation is yet to become a commercially available technology in India. This paper reports about the mobility, bioavaliability and plant response to presence of soil heavy metals. It classifies the plants according to phytoextraction mechanism and discusses the pathway of metal in plants. Various techniques to enhance phytoextraction and utilization of by-products have been elaborated. Since lot of biomass is produced during this process, it needs proper disposal and management. It also gives an insight into the work done by authors, which focuses on high biomass extractor plants. High biomas weeds were selected to restrict the passage of contaminants into the food chain by selecting non-edible, disease resistant and tolerant plants, which can provide renewable energy. Thus making phytoextraction more viable for present utilization.
Keywords. *heavy metals, phytoextraction, hyperaccumulator, indicator, excluder species*

Introduction

A major environmental concern due to dispersal of industrial and urban wastes generated by human activities is the contamination of soil. Controlled and uncontrolled disposal of waste, accidental and process spillage, mining and smelting of metalliferous ores, sewage sludge application to agricultural soils are responsible for the migration of contaminants into non-contaminated sites as dust or leachate and contribute towards contamination of our ecosystem. A wide range of inorganic and organic compounds cause contamination, these include heavy metals, combustible and putriscible substances, hazardous wastes, explosives and petroleum products. Major component of inorganic contaminates are heavy metals [1,2] they present a different problem than organic contaminants. Soil microorganisms can degrade organic contaminants, while metals need immobilisation or physical removal. Although many metals are essential, all metals are toxic at higher concentrations, because they cause oxidative stress by formation of free radicals. Another reason why metals may be toxic is that they can replace essential metals in pigments or enzymes disrupting their function [3]. Thus, metals render the land unsuitable for plant growth and destroy the biodiversity.

Though several regulatory steps have been implemented to reduce or restrict the release of pollutants in the soil, they are not sufficient for checking the contamination.

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Metal contaminated soil can be remediated by chemical, physical and biological techniques. These can be grouped into two categories [4].

Ex-situ method

It requires removal of contaminated soil for treatment on or of site, and returning the treated soil to the resorted site. The conventional ex-situ methods applied for remediating the polluted soils relies on excavation, detoxification and/or destruction of contaminant physically or chemically, as a result the contaminant undergo stabilisation, solidification, immobilisation, incineration or destruction.

In-situ method

It is remediation without excavation of contaminated site. Reed et al. defined in-situ remediation technologies as destruction or transformation of the contaminant, immobilisation to reduce bioavailability and separation of the contaminant from the bulk soil [5]. In-situ techniques are favoured over the ex-situ techniques due to their low cost and reduced impact on the ecosystem. Conventionally, the ex-situ technique is to excavate soil contaminated with heavy metal and their burial in landfill site [6, 7]. But the offsite burial is not an appropriate option because it merely shifts the contamination problem elsewhere [7] and also because of hazards associated with the transport of contaminated soil [8]. Diluting the heavy metal content to safe level by importing the clean soil and mixing with the contaminated soil can be an alternative of on-site management [9]. On-site containment and barriers provide an alternative, it involves covering the soil with inert material [10]. Immobilization of inorganic contaminant can be used as a remedial method for heavy metal contaminated soils [11]. This can be achieved by complexing the contaminants, or through increasing the soil pH by liming [12]. Increased pH decreases the solubility of heavy metals like Cd, Cu, Ni and Zn in soil. Although the risk of potential exposure to plants is reduced, their concentration remains unchanged. Most of these conventional remediation technologies are costly to implement and cause further disturbance to the already damaged environment [11,12]. Plant based bioremediation technologies have been collectively termed as phytoremediation, this refers to the use of green plants and their associated micro biota for the in-situ treatment of contaminated soil and ground water [13]. The idea of using metal accumulating plants to remove heavy metals and other compounds was first introduced in 1983, but the concept has actually been implemented for the past 300 years [3]. The generic term 'Phytoremediation' consists of the Greek prefix phyto (plant), attached to the Latin root remedium (to correct or remove an evil) [14]. This technology can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or the air [15,16]. The physico-chemical techniques for soil remediation render the land useless for plant growth as they remove all biological activities, including useful microbes such as nitrogen fixing bacteria, mycorrhiza, fungi, as well as fauna in the process of decontamination [17]. The conventional methods of remediation may cost from $10 to 1000 per cubic meter. Phytoextraction costs are estimated to be as low as $ 0.05 per cubic meter [18]. Phytoremediation consists of five main processes, shown in Table 1. This paper focuses studies on the phytoremediation especially phytoextraction of heavy metal contaminated soil using in-situ technique.

Rhizofiltration

It is defined as the use of plants, both terrestrial and aquatic; to absorb, concentrate, and precipitate contaminants from polluted aqueous sources with low contaminant concentration in their roots. Rhizofiltration can partially treat industrial discharge, agricultural runoff, or acid mine drainage. It can be used for lead, cadmium, copper, nickel, zinc and chromium, which are primarily retained with in the roots [19,20]. The advantages of rhizofiltration include it ability to be used as in-situ or ex-situ applications and species other than hyperaccumulators can also be used. Plants like sunflower, indian mustard, tobacco, rye, spinach and corn have been studied for their ability to remove lead from effluent, with sunflower having the greatest ability. Indian mustard has proven to be effective in removing a wide concentration range of lead (4 – 500 mg/l) [21]. The technology has been tested in the field with uranium (U) contaminated water at concentrations of 21-874 µg/l; the treated U concentration reported by Dushenkov was < 20 µg/l before discharge into the environment [22].

Table 1. *Phytoremediation includes the following processes and mechanisms of contaminant removal*

No.	Process	Mechanism	Contaminant
1.	Rhizofiltration	Rhizosphere accumulation	Organics/Inorganics
2.	Phytostabilisation	Complexation	Inorganics
3.	Phytoextraction	Hyper-accumulation	Inorganics
4.	Phytovolatilization	Volatilisation by leaves	Organics/Inorganics
5.	Phytotransformation	Degradation in plant	Organics

Phytostabilisation

It is mostly used for the remediation of soil, sediment and sludges [20,23] and depends on roots ability to limit contaminant mobility and bioavalability in the soil. Phytostabilisation can occur through the sorption, precipitation, complexaction, or metal valence reduction. The plants primary purpose is to decrease the amount of water percolating through the soil matrix, which may result in the formation of hazardous leachate and prevent soil erosion and distribution of the toxic metal to other areas. A dense root system stabilizes the soil and prevents erosion [24]. It is very effective when rapid immobilisation is needed to preserve ground and surface water and disposal of biomass is not required. However the major disadvantage is that, the contaminant remains in soil as it is, and therefore requires regular monitoring

Phytoextraction

It is the best approach to remove the contamination primarily from soil and isolate it, without destroying the soil structure and fertility. It is also referred as phytoaccumulation [20]. As the plant absorb, concentrate and precipitate toxic metals and radionuclide from contaminated soils into the biomass, it is best suited for the remediation of diffusely polluted areas, where pollutants occur only at relatively low concentration and superficially [25]. Several approaches have been used but the two basic strategies of phytoextraction, which have finally developed are; i) Chelate assisted phytoextraction or induced phytoextraction, in which artificial chelates are added to increase the mobility and uptake of metal contaminant. ii) Continuous phytoextraction in this the removal of metal depends on the natural ability of the plant to remediate; only the number of plant growth repetitions are controlled [26, 27]. Discovery of hyperaccumulator species has further boosted this technology. In order to make this technology feasible, the plants must, extract large concentrations of heavy metals into their roots, translocate the heavy metals to surface biomass, and produce a large quantity of plant biomass. The removed heavy metal can be recycled from the contaminated plant biomass [28]. Factors such as growth rate, element selectivity, resistance to disease, method of harvesting, are also important [29, 30]. However slow growth, shallow root system, small biomass production, final disposal limit the use of hyperaccumulator species [31]. Phytoextraction studies of Heavy metals have been elaborately discussed later.

Phytovolatilization

Phytovolatilization involves the use of plants to take up contaminants from the soil, transforming them into volatile form and transpiring them into the atmosphere. Phytovolatilization occurs as growing trees and other plants take up water and the organic and inorganic contaminants. Some of these contaminants can pass through the plants to the leaves and volatilise into the atmosphere at comparatively low concentrations [23]. Phytovolatilization has been primarily used for the removal of mercury, the mercuric ion is transformed into less toxic elemental mercury. The disadvantage is, mercury released into the atmosphere is likely to be recycled by precipitation and then redeposit back into ecosystem [3]. Gary Banuelos of USDS's Agricultural Research Service have found that some plants grow in high Selenium media produce volatile selenium in the form of dimethylselenide and dimethyldiselenide [32]. Phytovolatilization has been successful in tritium (3H), a radioactive isotope of hydrogen, it is decayed to stable helium with a half-life of about 12 years reported Dushenkov [33]

Phytodegradation

In phytoremediation of organics, plant metabolism contributes to the contaminant reduction by transformation, break down, stabilisation or volatilising contaminant compounds from soil and groundwater. Phytodegradation is the breakdown of organics, taken up by the plant to simpler molecules that are incorporated into the plant tissues [19]. Plants contain enzymes that can breakdown and convert ammunition wastes, chlorinated solvents such as trichloroethylene and other herbicides. The enzymes are usually dehalogenases, oxygenases and reductases [34]. Rhizodegradation is the breakdown of organics in the soil through microbial activity of the root zone (rhizosphere) and is a much slower process than phytodegradation. Yeast, fungi, bacteria and other microrganisms consume and digest organic substances like fuels and solvents. All phytoremediation technologies are not exclusive and may be used simultaneously, but the metal extraction depends on its bio available fraction in soil. The advantages and disadvantages have been discussed in Table 2.

Total and Bio-available fraction of Heavy Metals in soil

Heavy metals are elements having atomic weight between 63.54 and 200.59, and a specific gravity greater than 4 [35]. Trace amount of some heavy metals are required by living organisms, however any excess amount of these metals can be detrimental to the organisms [36]. Nonessential Heavy metals include arsenic, antimony, cadmium, chromium, mercury, lead, etc; these metals are of particular concern to surface water and soil pollution [35]. Heavy metals exist in colloidal, ionic, particulate and dissolved phase. Metals also have a high affinity for humic acids, organo clays, and oxides coated with organic matter [37,38]. The soluble forms are generally ions or unionised organometallic chelates or complexes. The solubility of metals in soil and groundwater is predominantly controlled by pH [3,4,6] amount of metal [39], cation exchange capacity [40], organic carbon content [37], the oxidation state of the mineral components, and the redox potential of the system [38]. In general, soil pH seems to have the greatest effect of any single factor on the solubility or retention of metals in soils. With a greater retention and lower solubility of metal cations occurring at high soil pH [41]. Under the neutral to basic conditions typical of most soils, cationic metals are strongly adsorbed on the clay fractions and can be adsorbed by hydrous oxides of iron, aluminium, or manganese present in soil minerals. Elevated salt concentration creates increased competition between cations and metals for binding sites. Also competitive adsorption between various metals has been observed in experiments involving various solids with oxide surfaces, in several experiments, Cd adsorption was decreased by the addition of Pb or Cu [42].

Table 2. *Advantages and disadvantages of phytoremediation.*

No	Advantages	Disadvantages / Limitations
1	Amendable to a variety of organic and inorganic compounds	Restricted to sites with shallow contamination within rooting zone of remediative plants.
2	*In Situ* / *Ex Situ* Application possible with effluent/soil substrate respectively.	May take up to several years to remediate a contaminated site.
3	*In Situ* applications decrease the amount of soil disturbance compared to conventional methods.	Restricted to sites with low contaminant concentrations.
4	Reduces the amount of waste to be landfilled (up to 95%), can be further utilized as bio-ore of heavy metals.	Harvested plant biomass from phytoextraction may be classified as a hazardous waste hence disposal should be proper.
5	*In Situ* applications decrease spread of contaminant via air and water.	Climatic conditions are a limiting factor
6	Does not require expensive equipment or highly specialized personnel.	Introduction of nonnative species may affect biodiversity
7	In large scale applications the potential energy stored can be utilized to generate thermal energy.	Consumption/utilization of contaminated plant biomass is a cause of concern.

Plant response to heavy metals

Plants have three basic strategies for growth on metal contaminated soil [16]; see Figure1.

Metal excluders

They prevent metal from entering their aerial parts or maintain low and constant metal concentration over a broad range of metal concentration in soil, they mainly restrict metal in their roots. The plant may alter its membrane permeability, change metal binding capacity of cell walls or exude more chelating substances [43].

Metal indicators

Species which actively accumulate metal in their aerial tissues and generally reflect metal level in the soil. They tolerate the existing concentration level of metals by producing intracellular metal binding compounds (chelators), or alter metal compartmentalisation pattern by storing metals in non-sensitive parts.

Metal accumulator plant species

They can concentrate metal in their aerial parts, to levels far exceeding than soil. Hyperaccumulators are plants that can absorb high levels of contaminants concentrated either in their roots, shoots and/or leaves [16,29,30]. Baker and Brooks have defined metal hyperaccumulator as plants that contain more than or up to 0.1% i.e. more than (1000 mg/g) of copper, cadmium, chromium, lead, nickel cobalt or 1% (>10,000 mg/g) of zinc or manganese in the dry matter. For cadmium and other rare metals, it is > 0.01% by dry weight [44]. Researchers have identified hyperaccumulator species by collecting plants from the areas where soil contains greater than usual amount of metals as in case of polluted areas or geographically rich in a particular element [45]. Approximately 400 hyperaccumulator species from 22 families have been identified. The *Brassicaceae* family contains a large number of hyperaccumulating species with widest range of metals, these include 87 species from 11 genera [44].

Figure 1. *Conceptual response strategies of metal concentrations in plant tops in relation to increasing total metal concentrations in the soil*

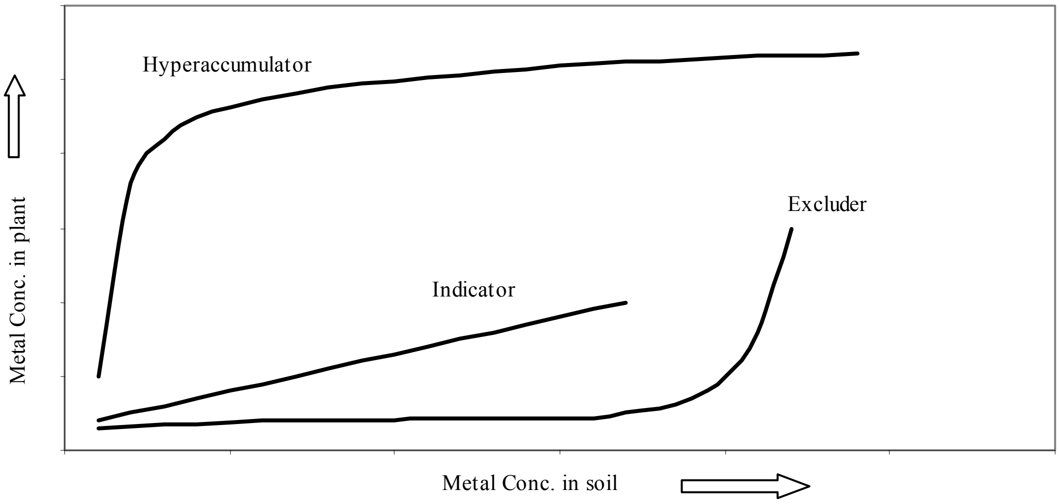

Figure. 1 - Conceptual response Strategies of metal concentrations in plant tops in relation to increasing total metal concentrations in the soil

Mechanism of Phytoextraction

The metal must mobilise into the soil solution, for the plants to accumulate metals from soil. The bioavalability of metals is increased in soil through several means. One-way plants achieve it by secreting phytosidophores into the rhizosphere to chelate and solublise metals that are soil bound [46]. Both acidification of the rhizosphere and

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exudation of carboxylates are considered potential targets for enhancing metal accumulation. Following mobilization, a metal has to be captured by root cells. Metals are first bound by the cell wall, it is an ion exchanger of comparatively low affinity and low selectivity. Transport systems and intracellular high-affinity binding sites then mediate and drive uptake across the plasma membrane. Uptake of metal ions is likely to take place through secondary transporters such as channel proteins and/or H+- coupled carrier proteins. The membrane potential, that is negative on the inside of the plasma membrane and might exceed –200 mV in root epidermal cells provides a strong driving force for the uptake of cations through secondary transporters [47].

Once inside the plant, most metals are too insoluble to move freely in the vascular system, so they usually form carbonate, sulphate or phosphate precipitates immobilizing them in apoplastic (extracellular) and symplastic (intra cellular) compartments [48]. Unless the metal ion is transported as a non-cationic metal chelate, apoplastic transport is further limited by the high cation exchange capacity of cell walls [48]. The apoplast continuum of the root epidermis and cortex is readily permeable for solutes. Apoplastic pathway is relatively unregulated, because water and dissolved substance can flow and diffuse without having to cross a membrane. The cell walls of the endodermal cell layer act as a barrier for apoplastic diffusion into the vascular system.

In general, solutes have to be taken up into the root symplasm before they can enter the xylem [49]. Subsequent to metal uptake into the root symplasm, three processes govern the movement of metals from the root into the xylem: sequestration of metals inside root cells, symplastic transport into the stele and release into the xylem. The transport of ions into the xylem is generally a tightly controlled process mediated by membrane transport proteins. Symplastic transport of heavy metals probably takes place in the xylem after they cross the casparian strip. It is more regulated due to the selectively permeable plasma membrane of the cells that control access to the symplast by specific or generic metal ion carriers or channels [50]. Symplastic transport requires that metal ions move across the plasma membrane, which usually has a large negative resting potential of approximately 170 mV (negative inside the membrane). This membrane potential provides a strong electrochemical gradient for the inward movement of metal ions. Most metal ions enter plant cells by an energy dependent saturable process via specific or generic metal ion carriers or channels [51].

Non-essential heavy metals may effectively compete for the same transmembrane carriers used by essential heavy metals. Toxic heavy metals such as cadmium may effectively compete for the same transmembranic carrier as used by micronutrient heavy metal. This relative lack of selectivity in transmembrane ion transport may partially explain why non-essential heavy metals can enter cells, even against a concentration gradient. For example, kinetic data demonstrate that essential Cu^{2+} and Zn^{2+} and non-essential Ni^{2+} and Cd^{2+} compete for the same transmembrane carrier [52]. Metal chelate complexes may also be transported across the plasma membrane via specialized carriers, as is the case for Fe–phytosiderophore transport in graminaceous species [53]. After heavy metals have entered the root they are either stored in the root or translocated to the shoots. Metal ions can be actively transported across the tonoplast as free ions or as metal–chelate complexes [54]. It is believed that in order to pass through the casparian strip, water and dissolved ions (salt and metal) require active transport, by utilising energy. For example, Cd is actively transported across the tonoplast of oat roots as either a free ion via a Cd/H^{+} antiport [55]. The vacuole is an important component of the metal ion storage where they are often chelated either by organic acid or phytochelatins. Insoluble precipitates may form under certain conditions. Precipitation compartmentalisation and chelating are the most likely major events that take place in resisting the damaging effects of metals [56]. Transporters mediate uptake into the symplast, and distribution with in the leaf occurs via the apoplast or the symplast [57]. Plants transpire water to move nutrients from the soil solution to leaves and stems, where photosynthesis occurs. Willows, hybrid poplar are also good phytoremediators, because they take up and process large volumes of soil water. For example, data show that a single willow tree, on a hot summer day, can transpire more than 19,000 litres of water [58].

Types of Phytoextraction

Natural Phytoextraction

In the natural setting, certain plants have been identified which have the potential to uptake heavy metals. At least 45 families have been identified to have hyperaccumulate plants; some of the families are *Brassicaceae, Fabaceae, Euphorbiaceae, Asteraceae*, *Lamiaceae*, and *Scrophulariaceae* [15,33]. Among the best-known hyperaccumulators is Thlaspi caerulescens commonly known as alpine pennycress [59], without showing injury it accumulated up to 26,000 mg kg^{-1} Zn; and up to 22% of soil exchangeable Cd from contaminated site [60,61]. Brassica juncea, commonly called indian mustard, has been found to have a good ability to transport lead from the roots to the shoots. The phytoextraction coefficient for *Brassica juncea* is 1.7 and it has been found that a lead concentration of 500 mg/l is not phytotoxic to *Brassica* species [3]. Phytoextraction coefficient is the ratio of the metal concentration found within the surface biomass of the plant over the metal concentration found in the soil. Some calculations indicate that Brassica juncea is capable of removing 1,1550 kg of lead per acre [3].

On a worldwide basis, concentrations > 1000 mg kg-1 are known for Ni in more than 320 plant species (sps.), Co (30 sps.), Cu (34 sps.), Se (20 sps.), Pb (14 sps.) and Cd (one sp.). The species involved in hyperaccumulation have recently been tabulated by Reeves and Baker [63], substantial number of these species are from Congo and Zaire. Concentration exceeding 10,000 mg kg-1 has been recorded for Zn (11 sps.) and Mn (10 sps.). The hyperaccumulation threshold levels of these elements have been set higher because their normal range in plants (20 – 500 mg kg^{-1}) are much higher than for the other heavy metals [62]. Aquatic plants such as the floating *Eichhornia crassipes* (water hyacinth), *Lemna minor* (duckweed), and *Azolla pinnata* (water velvet) have been investigated for use in rhizofiltration, phytodegradation, and phytoextraction [27]. Farago and Parsons [64] reported the bioremoval of platinum using *Eichhornia crassipes*. Many aquatic plants are used in the bioremoval of heavy metals e.g. *Azolla filliculoides*, *A. pinnata*, *Typha orientalis* and *Salvinia molesta*. Jin-Hong et al. in their study of twelve wetland species reported, *Polygonum hydropiperoides* Michx (smartweed) as the best for heavy metal phytoremediation, due to its faster growth and high plant density [65]. Recently, a fern *Pteris vitatta* has been shown to accumulate as much as 14,500 mg kg^{-1} arsenic in fronds without showing symptoms of toxicity [66].

Induced Phytoextraction or Chelate assisted Phytoextraction

Within the plant cell heavy metal may trigger the production of oligopeptide ligands known as phytochelatins (PCs) and metallothioneins (MTs) [67]. These peptides bind and form stable complex with the heavy metal and thus neutralise the toxicity of the metal ion [68]. Phytochelatin (PCs) is synthesised with glutathione as building blocks resulting in a peptide with structure Gly-(γ-Glu-Cys-)n; {where, n = 2-11}. Appearance of phytochelating ligands has been reported in hundreds of plant species exposed to heavy metals [69]. Metallothioneins (MTs), are small gene encoded, Cys-rich polypeptides. PCs are functionally equivalent to MTs [68].

Chelators have been isolated from plants that are strongly involved in the uptake of heavy metals and their detoxification. Chelating agents like ethylenediamine tetra acetic acid (EDTA) are applied to Pb contaminated soils that increases the amount of bioavailable lead in the soil and a greater accumulation in plants is observed [70]. The addition of chelates to a lead contaminated soil (total soil Pb 2500 mg kg–1) increased shoot lead concentration of *Zea mays* (corn) and *Pisun sativum* (pea) from less than 500 mg kg-1 to more than 10,000 mg kg^{-1}. This was achieved by adding synthetic chelate EDTA to the soil, similar results using citric acid to enhance uranium uptake have been documented. These results indicate that chelates enhanced or facilitated Pb transport into the xylem, and increased lead translocation from roots to shoots. For the chelates tested, the order of effectiveness in increasing Pb desorption from the soil was EDTA > Hydroxyethylethylene-diaminetriacetic acid (HEDTA) > Diethylenetriaminepenta-acetic acid (DTPA) > Ethylenediamine di(o-hyroxyphenylacetic acid) EDDHA [70]. Vassil et al., [71] reported that Brassica juncea exposed to Pb and EDTA in hydroponic solution was able to accumulate up to 55 mM kg^{-1} Pb in dry shoot tissue (1.1% [w/w]). This represents a 75-fold concentration of lead in shoot over that in solution. A threshold conc. of EDTA (0.25 mM) was required to stimulate this dramatic accumulation of both lead and EDTA in shoots.

Genetic Engineering to improve phytoremediation

To breed plants having superior phytoremediation potential with high biomass production can be an alternative to improve phytoremediation. General plant productivity is controlled by many genes and difficult to promote by single gene insertion. Genetic engineering techniques to implant more efficient accumulator gene into other plants have been suggested by many authors [29,60,72]. Implanting more efficient accumulator genes into other plants that are taller than natural plants increases the final biomass. Zhu et al. [73] genetically engineered Brassica juncea to investigate rate-limiting factors for glutathione and phytochelatin production; they introduced the Escherichia coli –gshl- gene. The γ-ECS transgenic seedlings showed increased tolerance to cadmium and had higher concentrations of Phytochelatins, γ-GluCys, glutathione, and total nonprotein thiols compared to wild type seedlings. The potential of success of genetic engineering can be limited because of anatomical constraints [74].

Limitations of Phytoextraction

Phytoextraction and plant-assisted bioremediation is most effective if soil contamination is limited to within 3 feet of the surface, and if groundwater is within 10 feet of the surface [16, 18]. It is applicable to sites with low to moderate soil contamination over large areas, and to sites with large volumes of groundwater with low levels of contamination that have to be cleaned to low (strict) standards [26]. This necessitates soil fertilization, conditioning, importance of employing effective agronomic practices [70, 72]. Scientists have investigated the effect of soil acidification on Zn and Cd phytoextraction and proposed the use of (NH4)2SO4 as a soil additive to

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provide nutrients (N and S) needed for high yield, and to acidify the soil for greater metal bioavailability. However, there might be some negative side effects associated with soil acidification. For example, due to increased solubility some toxic metals may leach into the groundwater creating an additional environmental risk. Chaney et al. [72] indicated that following metal phytoextraction, soil could be limed to elevate the pH near a neutral value, so that normal farm uses or ecosystem development could resume. However, premature liming may increase soil capacity for metal binding and restrict the potential for phytoextraction. A similar effect can be expected following the addition of organic fertilizers [69]. Phosphorus is a major nutrient, and plants respond favorably to the application of phosphate fertilizer by increasing biomass production [71]. The addition of these fertilizers, however, can also inhibit the uptake of some major metal contaminants, such as Pb, due to metal precipitation as pyromorphite and chloropyromorphite [72]. Natural chelators of plants or microbial origin seem more promising than synthetic chemical chelators [69]. It is uncertain whether an approach based on chemical chelators is practical for improving phytoextraction, since chemical chelators have additional toxicity to plants, thus they may increase the uptake of metals but decrease plant growth thus proving to be of limited benefit.

Utilization of Phytoremediation by-product

Phytoextraction involves repeated cropping of plants in contaminated soil, until the metal concentration drops to acceptable level. The ability of the plants to account for the decrease in soil metal concentrations as a function of metal uptake and biomass production plays an important role in achieving regulatory acceptance. Theoretically, metal removal can be accounted by determining metal concentration in plant, multiplied by the biomass produced; and comparing this with the reduction in soil metal concentrations. Although this sounds simple, many factors make it challenging in the field. One of the hurdles for commercial implementation of phytoextraction has been the disposal of contaminated plant material. After each cropping, the plant is removed from the site; this leads to accumulation of huge quantity of hazardous biomass. This hazardous biomass should be stored or disposed appropriately so that it does not pose any risk to the environment.

Biomass is nothing but stored solar energy in plant mass, it is also termed as materials having combustible organic matter. Biomass contains carbon, hydrogen and oxygen, it is known as oxygenated hydrocarbons. Biomass (specially wood) can be represented by the chemical formula $CH_{1.44}O_{0.66}$ [75]. The main constituents of any biomass material are lignin, hemicellulose, cellulose, mineral matter and ash. It possesses high moisture and volatile matter constituents, low bulk density and calorific value. The percentage of these components varies from species to species. The dry weight of Brassica juncea for induced phytoextraction of lead amounts to 6 tonnes per hectare with 10,000 to 15,000 mg/kg of metal in dry weight [76]. Handling of huge quantity of this type of waste is a problem and hence need volume reduction [77].

Composting and compaction has been proposed as post harvest biomass treatment by some authors [48,78,79]. Leaching tests for the composted material showed that the composting process formed soluble organic compounds that enhanced metal (Pb) solubility. Studies carried out by Hetland et al.,[80] showed that composting can significantly reduce the volume of harvested biomass, however metal contaminated plant biomass would still require treatment prior to disposal. Total dry weight loss of contaminated plant biomass by compaction is advantageous, as it will lower cost of transportation to a hazardous waste disposal facility. Compaction of harvested plant material was proposed by Blaylock and Huang [77] for processing metal rich phytoextraction residue. Advantages of compaction are similar as composting, the leachate will need to be collected and treated appropriately; in comparison to composting there is little information on compaction. One of the conventional and promising routes to utilize biomass produces by phytoremediation in an integrated manner is through thermochemical conversion process. If phytoextraction could be combined with biomass generation and its commercial utilization as an energy source, then it can be turned into profit making operation and the remaining ash can be used as bio-ore [28], this is also the basic principle of phytomining. Nicks and Chambers [81], reported a second potential use for hyperaccumulator plants for economic gain in the mining industry. This operation, termed phytomining includes the generation of revenue by extracting saleable heavy metals produced by the plant biomass ash, also known as bio-ore.

Combustion and gasification are the most important sub routes for organized generation of electrical and thermal energy. Recovery of this energy from biomass by burning or gasification could help make phytoextraction more cost-effective. Thermochemical energy conversion best suits the phytoextraction biomass residue because it cannot be utilized in any other way as fodder and fertilizers. Combustion is a crude method of burning the biomass, but it should be under controlled conditions, whereby volume is reduced to 2–5 % and the ash can be disposed properly. This method of plant matter disposal is often mentioned by many authors [48, 82]. It will not favourable to burn the metal bearing hazardous waste in open, as the gases and particulates released in the environment may be detrimental; only the volume is reduced and the heat produced in the process is wasted. Gasification is the process through which biomass material can be subjected to series of chemical changes to yield clean and combustive gas at high thermal efficiencies. This mixture of gases called as producer gas and/or pyro-gas that can be combusted for generating thermal and electrical energy. The process of gasification of biomass in a gasifier is a complex phenomenon; it involves drying, heating, thermal decomposition (pyrolysis) and gasification, and combustion chemical reactions, which occurs simultaneously [75]. Hetland et al., [80] reported possibility of co-firing plant biomass with coal, the results suggested that ashing reduced the mass of lead contaminated plant material by over 90 % and partitioned lead into ash. It may be possible to recycle the metal residue from the ash, however there are no estimates of the cost or feasibility of such a process [48]. Future experiments should concentrate on development of combustion system and methods to recycle different metals from ash. The process destroys organic matter, releasing metals as oxides. The liberated metals remain in the slag, modern flue gas cleaning technology assures effective capture of the metal containing dust. Considering the other technologies for disposal this method is environment-friendly.

Bridgewater et al., [82] reported that pyrolysis is a novel method of municipal waste treatment that might also be used for contaminated plant material. Pyrolysis decomposes material under anaerobic conditions; there is no emission to the air. The final products are pyrolytic fluid oil and coke; heavy metals will remain in the coke, which could be used in smelter. Koppolu et al., [83] reported that 99% of the metal recovered in the product stream was concentrated in the char formed by pyrolysing the synthetic hyperaccumulator biomass used in the pilot scale reactor. The metal

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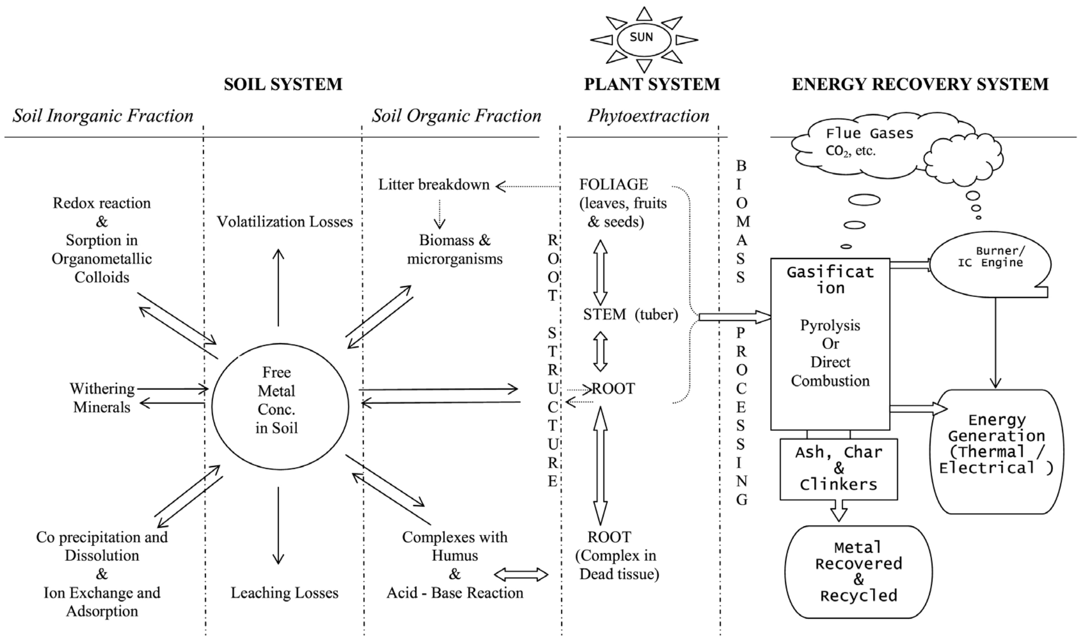
component was concentrated by 3.2–6 times in the char, compared to feed. Study of the fate of the metals in various feeds during pyrolysis has been addressed in literature in different context, but results on pyrolysis of phytoextraction plant biomass are limited. Helson et al., [84] conducted low temperature pyrolysis experiments with chromium, copper and arsenate treated wood and it was concluded that most of the metal was retained in the pyrolysis residue. Influence of metal ions on the pyrolysis of wood has been studied extensively by many authors [85, 86]

High cost of installation and operation can be a limiting factor for treatment if used solely for plant disposal. To avoid this plant material can be processed in existing facilities together with municipal waste. The authors worked on high biomass species, as they have shown positive result in screening (germination) studies [87]. The schematic diagram in Figure 2; describes the work of the authors on phytoextraction.

Figure 2. *The Soil, Plant and Energy Recovery System depicting the key components concerned with the mass transfer and dynamics of Phytoextraction*



The result of their work showed that phytoextraction of Cd, Cr and Pb by *Ipomoea carnea*, *Datura innoxia* and *Phragmytes karka* was higher in comparison to *Brassica juncea* and *Brassica campestris*, (known as indicator species) [3,88]. The study conducted with 10 to 200 mg kg^{-1} of Cd, Cr and Pb (separately) indicated that *I. carnea* was more effective in extracting them from soil than *B. juncea*. Among the five species, *B.juncea* accumulated maximum Cd but *I.carnea* followed by *D.innoxia* and *P.karka* were the most suitable species for phytoextraction of cadmium, if the whole plant or above ground biomass is harvested. In the relatively short time, *I.carnea* produced more than five times more biomass in comparison *B. juncea* [89]. It was more effective at translocating Cr from soil to plant shoot. *P.karka* showed much greater tolerance to chromium than other plants, though the uptake was low. Ipomoea extracted maximum lead at 200 mg kg^{-1}; *Datura* and *Phragmytes* was best extractor at 100 mg kg^{-1}, whereas Brassica species were at 50 mg Pb kg^{-1} soil [90]. Brassica species were difficult to cultivate, as they required pesticides to protect them from army moth, and secondly they cannot grow throughout the year. Whereas high biomass species do not have these limitations and showed higher potential, the extraction capacity can be further increased by use of chelates or soil additives.

Future of Phytoremediation

One of the key aspects to the acceptance of phytoextraction pertains to the measurement of its performance, ultimate utilization of by-products and its overall economic viability. To date, commercial phytoextraction has been constrained by the expectation that site remediation should be achieved in a time comparable to other clean-up technologies. So far, most of the phytoremediation experiments have taken place in the lab scale, where plants grown in hydroponic setting are fed heavy metal diets. While these results are promising, scientists are ready to admit that solution culture is quite different from that of soil. In real soil, many metals are tied up in insoluble forms, and they are less available and that is the biggest problem, said Kochian [59]. The future of phytoremediation is still in research and development phase, and there are many technical barriers which need to be addressed. Both agronomic management practices and plant genetic abilities need to be optimised to develop commercially useful practices. Many hyperaccumulator plants remain to be discovered, and there is a need to know more about their physiology [16]. Optimisation of the process, proper understanding of plant heavy metal uptake and proper disposal of biomass produced is still needed.

Conclusion

Phytoremediation is a fast developing field, since last ten years lot of field application were initiated all over the world, it includes Phytoremediation of Organic, Inorganic and Radionuclides. This sustainable and inexpensive process is fast emerging as a viable alternative to conventional remediation methods, and will be most suitable for a developing country like India. Most of the studies have been done in developed countries and knowledge of suitable plants is particularly limited in India. In India commercial application of Phytoremediation of soil Heavy metal or Organic compounds is in its earliest phase. Fast growing plants with high biomass and good metal uptake ability are needed. In most of the contaminated sites hardy, tolerant, weed species exist and phytoremediation through these and other non-edible species can restrict the contaminant from being introduced into the food web. However, several methods of plant disposal have been described but data regarding these methods are scarce. Composting and compaction can be treated as pre-treatment steps for volume reduction, but care should be taken to collect leachate resulting from compaction. Between the two methods that significantly reduce the contaminated biomass, incineration seems to be least time consuming and environmentally sound than direct burning or ashing.

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SEMICONTINUOUS CULTURE SYSTEM FOR *LEMNA GIBBA* BIOASSAY: FUNCTIONING AND THEORY OF OPERATION

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Abstract. To overcome the problems associated with high concentrations of nutrient and test chemicals in laboratory *Lemna gibba* bioassays, a semi-continuous culture system was adopted, and specific equipment was designed and constructed to simulate steady-state conditions of the most natural aquatic systems. The equipment for *Lemna* semicontinuous culture consists of a growth chamber and a circulation control unit. *Lemna gibba* is kept in the growth chamber in a growth pot (with 1 mm diameter perforation at about 10 cm above the bottom), which retains the plant but allows an efficient exchange of the growth medium. Flow rate and composition of the medium is therefore varied independently of the *L. gibba* population density. The media are kept in circulation, and recharges or discharge of the media are controlled. The system took into consideration chemical processes such as O_2 and CO_2 exchange and maintained a degree of turbulence through continuous shaking of the growth chambers. It is also possible to investigate growth under different supply of O_2 and CO_2, and pH control with dissolved CO_2 or reducing conditions with N_2 gases. Theoretical analysis of growth in the steady state shows that integrated activation time of the dilution pump is proportional to the growth rate of *L. gibba*. Theoretical analysis was also used to determine the minimum flow-rate and nutrient concentration of the medium to cover the requirements of *L. gibba*. Experiments were carried out that demonstrated that the steady state growth could be attained and be controlled by the nutrient concentration, flow rate, and recharge intervals. The cultures could be kept at steady state over 21 days of the *Lemna* test period.
Keywords. *Lemna gibba, steady state, semicontinuous culture, batch culture, nutrient media*

Introduction

The validity of results obtained from laboratory tests performed batch-wise are often questioned because of dissimilarities to natural systems in respect to physical characters as well as process conditions such as high nutrient and test substance concentrations [2, 17]. The need to ensure realistic concentrations in hydroponic culture tests has been recognised for some decades [2, 15]. Various strategies, e.g., recharge or buffer system, have been developed. One of such systems is continuous culture, which became widely used particularly for microbes, algae and cell culture for industrial and research purposes [1, 7, 10, 11, 30, 31]. The early development of the continuous culture system can be traced back to simultaneous works of Novick & Szilard [27] and Monod [25].

Continuous culture systems are described as: (1) steady-state, where nutrients are supplied to the culture at a constant rate, and a constant amount of biomass is maintained in the culture. Steady-state systems have constant metabolic and growth parameters. (2) Turbidostat, where medium is delivered only when the population density of the culture reaches some predetermined point, as measured by the extinction of space in the culture. Fresh medium is, then, added to the culture and an equal volume is removed [32]. (3) Chemostat, where the medium is delivered at a constant rate, which ultimately determines growth rate and density [5, 7, 27]. In order to deliver exactly the same amounts of medium to the cultures and reproducibility of the experiments, a steady-state approach through a semicontinuous culture mode is usually preferred [1, 7, 10]. Semicontinuous culturing differs from batch culture in that fresh medium is added to the culture at a regular rate and spent medium is removed at the same rate. The principal advantage is that tests are conducted with realistic concentrations emulating the nature of the nutrient buffering capacity [15].

In recent years, continuous culture techniques have found their way into the bioassay methods of ecotoxicology and bioremediation. However, their adoption and application for higher plant hydroponic cultures has been so limited because realisation of a system that circulates and re-charges large volumes while maintaining a low realistic concentration proved to be expensive [2, 3, 4]. Consequently, most test procedure like the standard *Lemna* tests use batch culture modes, despite the well publicised difference from natural conditions [12–14, 28, 29]. In *L. gibba* cultures, a semicontinuous culture mode reduces competition between algae and *Lemna* spp., reduces poisoning from metabolic excretion, and reduces large changes of chemical speciation in the solution [20, 33]. To investigate mechanisms that control processes in phytoremediation and ecotoxicity of uranium and arsenic to aquatic macrophytes, laboratory and field trials with the model plant *L. gibba* in a semicontinuous culture were opted. Hence, the semicontinuous culture equipment was designed for *L. gibba*, whose functioning and design are described in this paper.

General *Lemna gibba* growth conditions

The most important parameters regulating frond growth are nutrient quantity and quality, light, pH, turbulence, salinity, and temperature [6, 8, 12, 18, 19]. The optimal parameters as well as the tolerated ranges are species-specific, and a broad generalization for the most important parameters for *Lemna* spp. is given in *Table 1*. The *Lemna* semi-continuous culture has been designed to take into consideration as many parameters as possible while its optimum is reached when operated in plant chamber (ecotron).

***Table 1**. A generalized set of conditions for culturing L. gibba*

parameters	optimal range	source
Temperature (°C)	18–24 °C	ISO/WD 20079
Salinity (g l^{-1})	20–24 g l^{-1}	ISO/WD 20079
Light intensity (lux)	4200 and 6700 lux	EPA712–C–96–156 OPPTS 850.4400
	85–125 µE m^{-2} s^{-1} or 400–700 nm	ISO/WD 20079
Photoperiod	14–16 hr d^{-1}	own unpublished data
pH	5.6–7.5	Mkandawire *et al*. (2002)

Parts and set-up of the culture system equipment

The system is designed to maintain a steady state in the *L. gibba* culture. The system has 12 *Lemna* culture vessels composed of two parts: (1) the outer vessel and (2) an inner growth pot. The medium is circulated to the culture at a constant rate. The flow rate of the medium is adjusted according to the concentration of the medium and growth

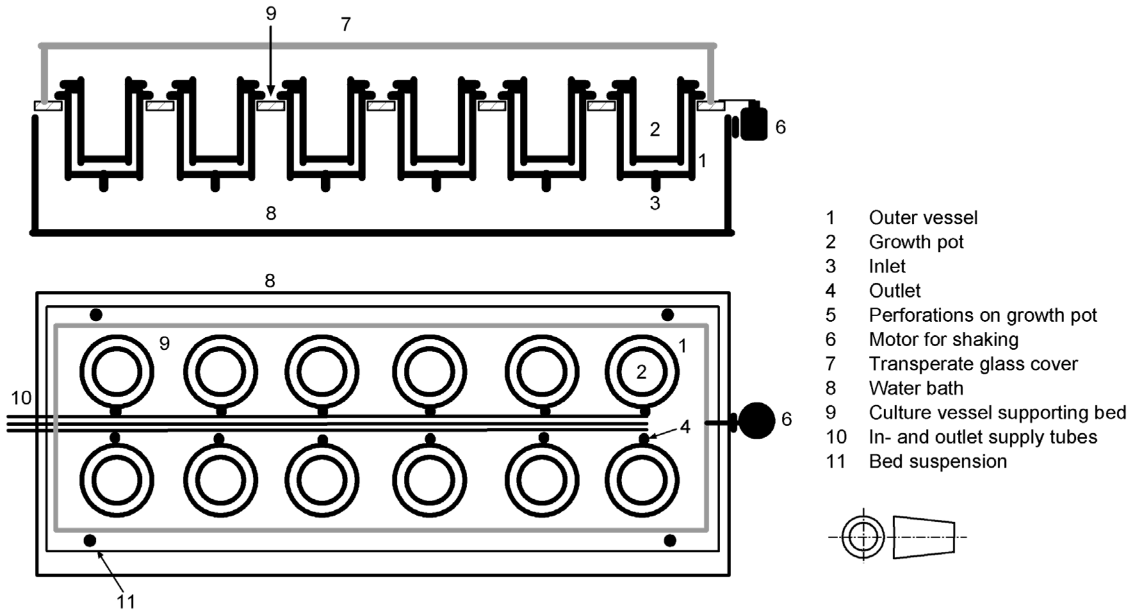

Figure 1. *Frond and plan in 1st angle orthographic projection view of the part set-up of the main semicontinuous culture unit*

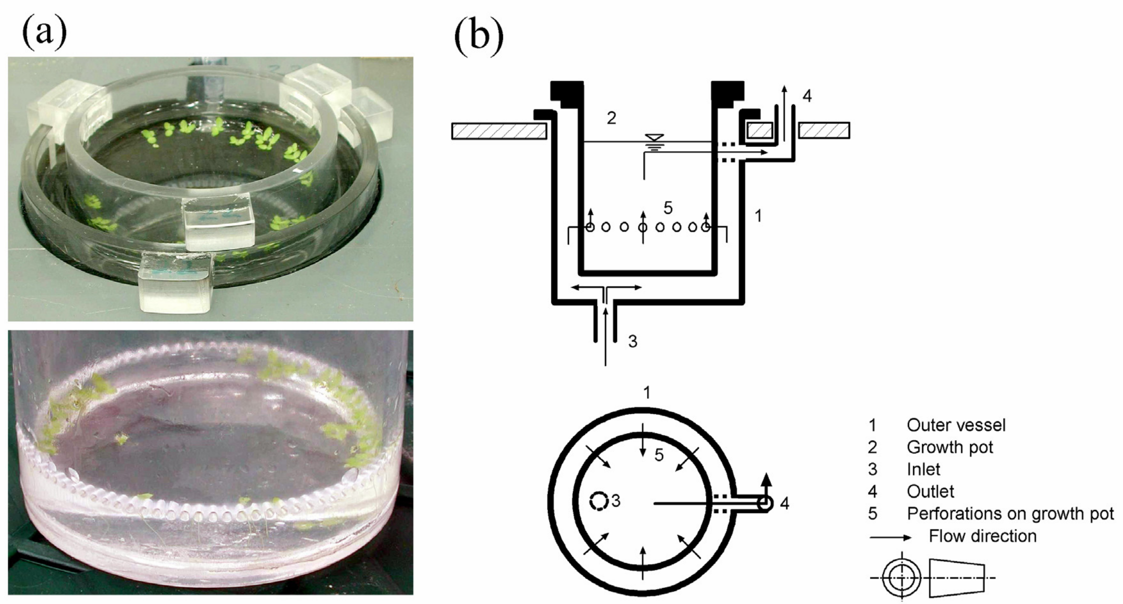

Figure 2. *Picture of growth vessel showing the perforation on the growth pot; and (b) front and plan view of Lemna growth vessels showing the circulation on the medium*

rate of *L. gibba*. If the growth rate is not well calculated or defined, the literature recommends that approximately 20% of culture should be removed as spent and replaced by an equal volume [10, 11, 16]. Each *Lemna* growth vessel has an inlet and outlet connection (3 and 4). The growth pot has perforation at 20 mm from the base and top to enable the flow of solutions. This also allows easy monitoring with minimum disturbances; i.e., once the growth pot is pulled out of the chamber, the *Lemna* specimen is left at a 20 mm depth of the medium, e.g., during growth observations with frond area with graphic image equipment.

The growth vessels are deep in a water bath (5) to equilibrate temperatures in the vessels (*Fig. 1*). The bed is suspended (9) and swings with the help of a motor (6), to a required turbulence. The turbulence or the continuous shaking of the growth vessels helps to maintain the solution well mixed, and exchange of gases with the air. The vessels are covered with (7) a transparent glass cover to avoid fallouts from the air.

The medium circulates from the vessel through the tube to filters, which helps reduce the growth of microorganisms like algae and remove dead fronds, and back to the culture vessel. Fresh nutrient medium is stored in a container (11). Opening a valve and closing the clamp on the medium line can add medium to the culture vessel. Fresh medium flows into the culture vessel, and the spent culture flows out into a collecting vessel. Optionally, the system allows working in reducing conditions by allowing a flow of N_2, and different O_2 and CO_2 regimes. As fresh medium is added to the culture vessel, the level of the liquid in the culture vessel rises to the level of the outflow tube and the old medium flows out of the culture vessel into a spent medium flask.

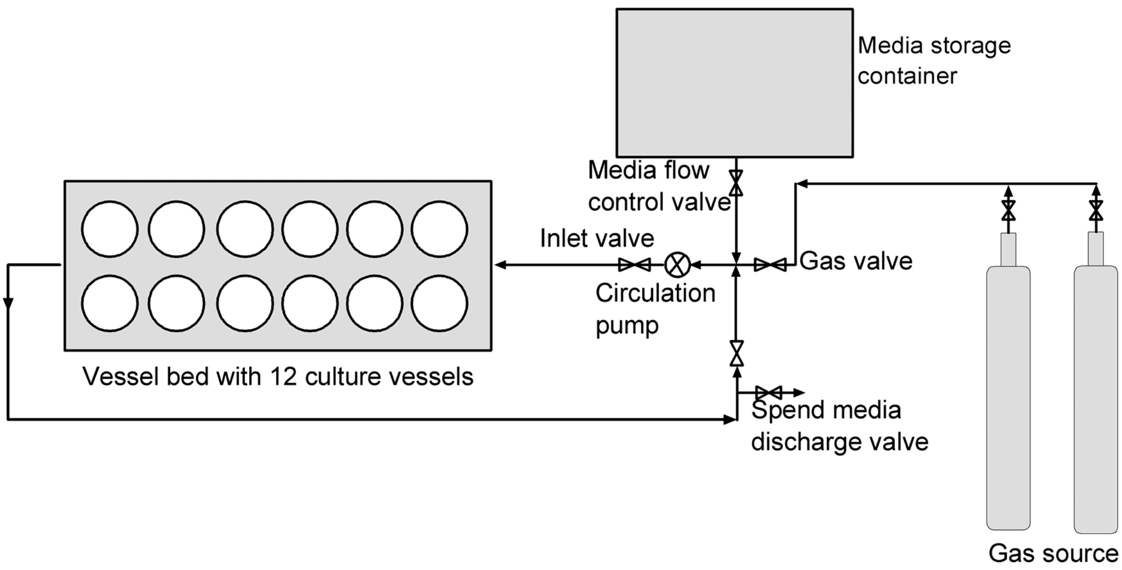

Figure 3. *Principle sketch of the functioning of the Lemna semicontinuous culture system*

Functioning of the *Lemna* semicontinuous culture system equipment

In a semi-continuous system, the fresh medium is delivered to the culture all at once, by simply opening a valve in the medium delivery line. Fresh medium flows into the culture vessel, and the spent culture flows out. Once the required medium has entered the culture, the valve is closed, and the culture is allowed to grow for a given period, when the procedure is repeated. The natural system is at steady state because there is continuous exchange of chemical elements and compounds between the geo- and hydrosphere. In preliminary *Lemna* bioassay studies with a batch system, it was found that the pH decreases as *L. gibba* grows [20, 24]. This happens partially because of release of CO_2 from plants respiration at night, exudation and the resources are depleted in the medium [20, 26]. Shaking creates turbulence in the system that helps release CO_2 from the culture, while at the same time there is dissolution of oxygen. The space between the inner growth pots at the outer vessel in figure 4.3 is for this purpose, particularly when the pot is fully covered by an *L. gibba* mat.

Function and operation theory

Experiments with *L. gibba* were conducted to parameterise the processes in the equipment as described elsewhere [20–23]. Performance of *L. gibba* changes in the culture physicochemical condition in batch and semicontinuous cultures have been reported in Mkandawire *et al.* [24]. The data read to the general theory as below.

Under steady state *L. gibba* growth, the growth is expected to be constant throughout the study, but practically the growth rate fluctuates between *a* and *c* around this arbitrary constant growth rate *b* in *Fig. 5*. The length is modulated by the concentration of the medium, which also determines the recharge interval P_n. From the experiment (data not shown), it was observed that the curves smooth when the recharge rate approaches the continuous; i.e., when the gaps from one nutrient recharge into the culture are close to mathematical zero.

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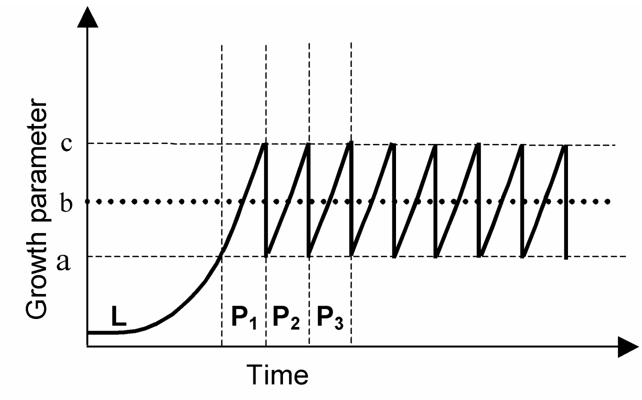

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Figures 4 and 5. 4: *The main part of the semicontinuous culture system for L. gibba bioassay.* 5: *Periodic fluctuation in steady state in L. gibba semicontinuous culture. L represents pre-culture period; P1, P2, ..., Pn are nutrient recharge and biomass removal intervals also shown as t_1,..., t_n in the text.*

The rate of flow of medium into a semicontinuous culture system is sometimes referred to as the dilution rate [9, 15]. When the number of fronds in the culture vessel remains constant over time, the dilution rate is equal to the rate of multiplication in the culture, because a known number of *L. gibba* fronds, removed in relation to the discharge of medium, are replaced by an equal number through multiplication in the culture The recharging interval time (t_n) (or the length of the period between the inflows of nutrients) in a semi-continuous culture is inversely related to the concentration of the nutrient medium. Assuming the undiluted solution medium (*N*) is the standard used in batch culture, plotting the dilution factor (*N*) against t_n shows that as t_n approaches zero, the system gradually becomes continuous. The relationship is a linear line regression as:

$$N = -kt_n + c\,, \qquad \text{(Eq. 1)}$$

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where k is the regression slop constant; c is a constant, x in N_m are integers 0, 1, 2, 3,…, m; and similarly n in t_n are 0, 1, 2, 3,…, n. Therefore, k becomes the flow rate (F) and at t_0 $N = N_m$ it can be rewritten as:

$$N = -Ft_n + N_m . \qquad \text{(Eq. 2)}$$

For simplicity, points on the regression are assumed to be the lowest concentration at which a plant is capable to attain its maximum specific growth rate (μ_{max}). The rate of flow (f) of medium into a semicontinuous culture system is directly related to the dilution rate (D), whereas D is inversely related to the volume of the medium (v_{med}):

$$D = \frac{f}{v_{med}} . \qquad \text{(Eq. 3)}$$

In this equipment D and f are not the same. Increase in the number of *L. gibba* fronds in the culture is proportional to the specific growth rate (μ) and the yield rate (E), i.e., the amount of *L. gibba* to be removed to maintain a steady state. When the steady state condition are met, the relationship in the dynamic equilibrium becomes as follows:

$$\frac{dx}{dt} = \mu X - DX = 0 \qquad \text{(Eq. 4)}$$

and

$$\frac{dx}{dt} = \mu X - E = 0 , \qquad \text{(Eq. 5)}$$

where x is the growth parameter, and t is the time. It follows that $\mu X = E$, and further analysis shows that X is constant, resulting in E being directly proportional to μ. This depends on the volume of the culture vessel and on the concentration (S) and further depends on the nutrient uptake by *L. gibba*, and Monod model can be adopted:

$$\mu = \mu_{\max} \frac{s}{s + k_g} ; \qquad \text{(Eq. 6)}$$

k_g is the saturation constant at which $\mu = \frac{1}{2}\mu_{\max}$. At D, $S = S_0$ which is theoretically valid even when considering single growth-limiting nutrient elements like N or P. For a small dilution rate, let the *L. gibba* volume ratio *(Z)* be expressed by

$$Z = \frac{X}{V} . \qquad \text{(Eq. 7)}$$

This allows that uptake

$$A_s = D(S_0 - S) . \qquad \text{(Eq. 8)}$$

Then,

$$\frac{\Delta D}{-\Delta S} = \frac{dx}{ds} = y , \qquad \text{(Eq. 9)}$$

where y is the economic quotient. In relationship to changes in the substrate concentration when steady state conditions are fulfilled,

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$$\frac{dS}{dt} = D(S_0 - S) - \mu_{max}\frac{X}{y}\frac{S}{k_s - S} = 0. \qquad \text{(Eq. 10)}$$

Considering the optimal range of substrate concentration where maximum growth rate is attained, uptake A_s can be given as

$$A_s = \mu_{max}\frac{X}{Y} = C, \qquad \text{(Eq. 11)}$$

where, C is a constant. Hence,

$$D(S_0 - S) = \mu_{max} \qquad \text{(Eq. 12)}$$

and

$$S = S_0 - \frac{\mu_{max}\frac{x}{y}}{D}. \qquad \text{(Eq. 13)}$$

In the suboptimal range, the substrate concentration depends on D; and, likewise,

$$D = \mu_{max}\frac{S}{K_s + S}. \qquad \text{(Eq. 14)}$$

Conclusion

The principal advantage of semicontinuous culturing is that the rate of dilution controls the growth rate (μ) via the concentration of the growth-limiting nutrient in the medium, found in *L. gibba* cultures to be phosphorus [21, 23]. As long as the dilution rate is lower than the maximum growth rate (μ_{max}) attainable by *L. gibba*, the frond density will increase to a point at which the frond multiplication rate exactly balances the removal and death rate. This steady-state frond density is also characterised by a constancy of all metabolic and growth parameters. On the other hand, if the dilution rate exceeds the maximum growth rate, fronds are removed faster than they are produced, and a total decrease in the entire frond population eventually occurs.

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CHARACTERISTICS OF ACID RAIN IN JINYUN MOUNTAIN, CHONGQING, CHINA

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Abstract. The pH and chemical composition of 126 precipitation samples, which were collected from April 1998 to November 1999 in Jinyun Mountain, Chongqing, China, were analyzed. The results showed: the average pH was 5.23 and the average electrical conductivity was 33.90 µs/cm, which showed that the pollution situation in Jinyun Mountain was more favourable than that in the other acid rain regions of southern China and Chongqing urban areas. The concentration of SO_4^{2-} accounted for 84.61% of total anions, and the concentration of NH_4^+ and Ca^{2+} accounted for 77.36% of total cations of rainfall in Jinyun Mountain. The high concentration of NH_4^+ and Ca^{2+} neutralized greatly the acidity of acid ions, which resulted in relatively high pH and lower frequency of acid rain, but the acidifying rain may exert potential injury to forest ecosystem. The seasonal variations in the rainfall pH and chemical composition were evident, which may be related to the seasonal variations in precipitation amount in Jinyun Mountain. The effects of acid rain on forest ecosystem have time lag, so it is necessary to study the dynamic characteristics of acid deposition more carefully and comprehensively.
Keywords. *pH , major ions, air pollution, acidifying rain*

Introduction

The air pollutants from burning the coal with high sulfur and high ash content without any treatment are the source of serious air pollution. Especially, Chongqing Municipality, which is located at Southwestern China, is well known as a typical area polluted by coal combustion and has suffered from serious air pollution and acid rain [6, 17, 19]. This situation in Chongqing downtown has been improved in recent years since some SO_2 emission control technologies have been developed, such as SO_2 emission by high chimneys, bio-briquette technique, the installation of the desulfurizing equipment, and so on [3, 4, 20]. But, in general, Chongqing still suffers from serious air pollution and acid rain, and the acid deposition has tended to extend from urban site to the suburb sites in recent years [3, 16, 22]. With the proceeding of Three Gorges Reservoir of Yangtze River, the government has paid more attention to the ecological and environmental problems including serious air and water pollution in Chongqing region. Thus, it is necessary to study the new dynamic characteristics of precipitation chemistry in Chongqing (especially, in the suburb regions of Chongqing) in order to protect the forest vegetation and ecological environment better.

The National Nature Reserve of Jinyun Mountain is located in Beibei district, 60 km north from Chongqing downtown. Having good vegetation and lacking heavy industry, Jinyun Mountain was thought to be free from serious air pollution and acid rain

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compared with the situations in Chongqing downtown and other urban areas (e.g. [1]). But, in recent years, the plant damage caused by acid rain has emerged in this region. The objective of our research in this experiment was to analyze the dynamic characteristics of precipitation chemistry and pH value in Jinyun Mountain in order to monitor those air pollutions and control the damage to forest ecosystem.

Materials and methods

Experiment site

Jinyun Mountain (29°49'N, 106°20'E) covers 1400 ha and the altitude is 350–952 m. The typical vegetation is sub-tropical broadleaved evergreen forest, which is the one of best-protected broadleaved evergreen forests in the Yangtze River basin. The plant species are abundant (over 1400 species), and the coverage of vegetation is over 90%. Some sensitive plant species in this region had been hazarded by acid rain in recent years.

Jinyun Mountain region belongs to sub-tropical humid monsoon climate zone. Annual mean temperature is 18.2°C, the frost-free period is 334 days and the annual hours of sunshine were 1288.1. The bulk of rain falls from April through October; the average annual precipitation is 1143 mm and the annual relative humidity is 80%. The main soil type is locally called yellow mountain soil, which corresponds to Haplic Acrisol in the FAO classification system.

Sampling and chemical analysis

126 rainfall samples were collected on a daily basis using a wet-only sampler in Jinyun mountain (alt. 500 m) from April 1998 to November 1999. The stored samples were sent in ice-cooled boxes to the laboratory at the foot of Jinyun Mountain. The analytical parameters were pH value, electrical conductivity and the concentrations of major ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, NH_4^+, SO_4^{2-}, NO_3^- and Cl^-). The pH value of each sample was measured by the pH meter with a glass electrode and conductivity was measured by a digital conductivity meter. In the samples, SO_4^{2-}, NO_3^- and Cl^- concentrations were measured by ion chromatography. K^+, Na^+, Ca^{2+} and Mg^{2+} concentrations were measured by atomic absorption spectrometry, and NH_4^+ concentration was measured by spectrophotometric indophenol method. Each data point was generated as an average of three measurements.

Data from monitoring acid rain in Jinyun Mountain were analyzed to characterize the pH value and chemical compositions of precipitation. The seasonal differences of pH and electrical conductivity were analyzed by the analysis of variance (ANOVA) and the relationships between different major ions were examined by the principal component analysis (PCA) and correlation analysis.

Results

The pH value and electrical conductivity

The general characteristics

The average pH value of 126 rainfall samples in Jinyun Mountain was 5.23. In comparison to the other regions, this value was lower than pH in Northern China, but was much higher than the mean pH in Southern China, the downtown and Nanshan Mountain in Chongqing urban (*Table 1*).

Although the average pH was higher than other typical acid rain areas in Chongqing, pH of individual rainfall in Jinyun Mountain showed a very large fluctuation, ranging

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Table 1. *The average pH value of precipitation in various regions of China*

China (north)[a]	China (south)[a]	Chongqing (downtown)[b]	Chongqing (Nanshan Mt.)[c]	Chongqing (Jinyun Mt.)
5.80	4.70	4.11	4.60	5.23

[a] [12], [b] [6], [c] [15].

from 3.80 to 7.30 (see *Fig. 1*). The number of samples below pH 5.6, the pH of unpolluted water equilibrated with atmospheric CO_2, was 77, accounted for 61.1% of total samples. About 0.8% of the rainfall showed a pH of lower than 4.0 and 44.4% had pH between 4.0 and 5.0. The frequency distribution of precipitation pH was similar to a bimodal distribution and the kurtosis is -1.079.

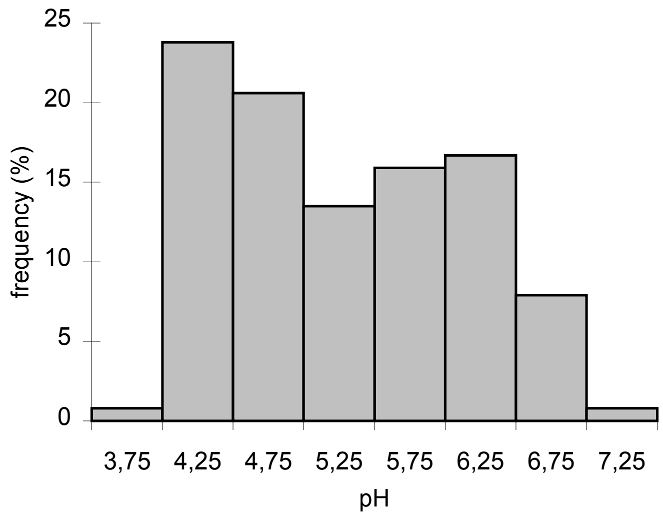

Figure 1. *The pH frequency distribution of precipitation in Jinyun Mountain*

The pH values of precipitation were not correlated directly with the concentrations of major ions, but were related to the neutralized effects of acid anions and alkaline cations [16, 20]. Thus, the single precipitation pH could not reflect the degree of precipitation pollution. The average conductivity of rainfall in Jinyun Mountain was 33.90 µs/cm (the maximum value was 131.0 µs/cm and the minimum value was 5.5 µs/cm), which significantly was lower than that in Chongqing downtown (73.5 µs/cm) [18]. That is to say, the situation of air and precipitation pollution in Jinyun Mountain were really much favourable than that in Chongqing urban areas.

Seasonal variation

Table 2 lists the results of seasonal differences of pH and electrical conductivity analyzed by ANOVA. The precipitation pH and conductivity showed pronounced seasonal variations. The average pH (4.24) in the winter was lower significantly than that in other three seasons and the frequency of acid rain in the winter was also significantly higher ($P<0.01$), which may be related with the seasonal variation of precipitation amount. The conductivity had maximum value in the winter and minimum value in the summer, which showed the situation of air and precipitation pollution in Jinyun Mountain were much severe in winter than in summer.

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Table 2. *The seasonal variation of precipitation pH and conductivity in Jinyun Mountain*

season	pH mean	pH min	pH max	conductivity (μs/cm)	frequency of acid rain (%)
spring	5.37±0.83[a]	4.00	6.55	39.52[a]	53.1
summer	5.17±0.77[a]	4.10	7.30	21.69[b]	65.1
autumn	5.36±0.82[a]	4.00	6.90	38.88[a]	50.0
winter	4.24±0.22[b]	3.80	4.50	56.81[a]	100.0

The different letter in the same column means the values have significant difference ($p<0.01$)

Major Ions

Anions

The acid rain in Jinyun Mountain was also classified to sulfuric acid type as in Chongqing urban areas and Southern China. Among all measured anions, SO_4^{2-} was the most abundant with an average of 212.85 μeq/l (see *Table 3*), which accounted for 84.61% of the total anions (*Fig. 2a*). The second most abundant anion was Cl^- with an average concentration of 24.70 μeq/l. The average NO_3^- concentration, 14.02 μeq/l, was lower than one tenth of SO_4^{2-} and accounted for 5.57% of the total anions.

The sum of all anions amounted to 288.45 μeq/l, which was much lower than those of the Chongqing urban areas (see *Table 3*), but considerably higher than those of the typical acid rain areas in Northeast American and Central Europe [6, 9, 10, 15]. If all these ions existed as free acid forms, the precipitation pH would have been lower than 5.23. This large discrepancy indicated that the acids present in the precipitation of Jinyun Mountain had gone through a significant neutralization process.

The concentration of NO_3^- was much lower than that of SO_4^{2-}. Thus, the ratio of the concentration of NO_3^- to SO_4^{2-} (N : S ratio), 0.07, was much lower than the value obtained from some developed countries [8, 9, 10, 11], which showed intensive influence of SO_2 from the coal burning and weak influence of NO_x from traffic on precipitation. But, with the development of vehicle-industry and living standard of people in China, emissions of NO_x are expected to increase rapidly and will become another important source of air pollution.

Cations

As seen in much of the monitoring data for precipitation in China, the difference among the developed countries in Northeast American and Central Europe resulted from high levels of cations, such as NH_4^+ and Ca^{2+} [6, 7]. The measured mean concent

Table 3. *The average ion composition of precipitation in various regions of Chongqing*

ion	downtown	Nanshan Mountain	Jinyun Mountain
K^+	17.0	82.6	18.15
Na^+	17.0	23.9	28.34
Ca^{2+}	125.0	418.0	80.78
Mg^{2+}	31.0	40.3	18.82
NH_4^+	123.0	106.0	142.37
SO_4^{2-}	299.0	469.0	212.85
NO_3^-	23.0	45.0	14.02
Cl^-	30.0	27.6	24.70
∑anions	352.0	541.6	251.57
∑cations	313.0	670.8	288.45
∑anions : ∑cations	1.12	0.81	0.87

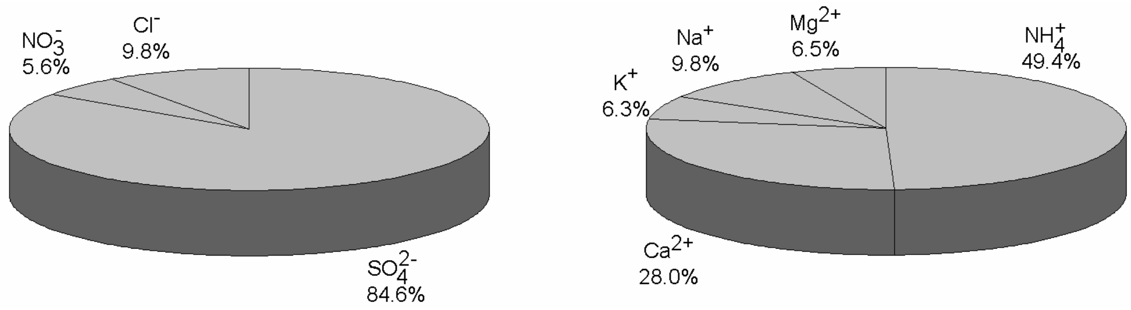

Figure 2. *The major ion composition of precipitation in Jinyun Mountain.* a: *anions;* b: *cations*

rations of NH_4^+ and Ca^{2+} in Jinyun Mountain were 142.37 μeq/l and 80.78 μeq/l respectively (see *Table 3*), which accounted for 49.36% and 28.00% of all cations respectively (see *Fig. 2b*). The NH_4^+ concentration was higher than that in Chongqing urban areas while the concentration of Ca^{2+} was much lower. The high level of NH_4^+ in precipitation coincides with level of ammonia emissions in the neighboring region of Jinyun Mountain. The Nature Reserve is surrounded by farm fields, thus the intensive fertilization and relatively poor treatment of waste from barns and houses might be the reasons for the higher levels of gaseous ammonia. The low level of Ca^{2+} in the precipitation may be related the high coverage of vegetation in Jinyun Mountain and it's neighboring areas which decreased the natural alkaline dust from roads and fields.

Statistical analysis

Relationships between measured major ions were examined through factor analysis and correlation analysis. The results analyzed by principal component analysis (PCA) by varimax rotation to a set of orthogonal axes for 1008 precipitation data were presented in *Table 4*.

Component 1 accounted for 57.6% of the total variance and had high loadings for K^+, Na^+, Mg^{2+}, Ca^{2+}, NH_4^+ and SO_4^{2-}. In component 2, NO_3^- was strongly loaded and accounted for 15.6% of the total variance. In component 3, Cl^- was loaded highest and accounted for 9.1% of total variance. The first three components accounted for 82.3% of total variance.

The correlations among major ions were presented in *Table 5*. The highest correlations appeared between every alkaline cation and SO_4^{2-}. Especially, the correlation coefficients between Ca^{2+} and SO_4^{2-}, NH_4^+ and SO_4^{2-} were 0.752 and 0.735 ($P<0.01$), respectively, revealing their co-occurrence in precipitation mostly as $CaSO_4$, $(NH_4)_2SO_4$, $(NH_4)HSO_4$ and so on. In other words, SO_4^{2-} ions in the precipitation were present mostly as neutralized forms and significantly decreased the precipitation acidity, which reduced the direct damage of acid rain to forest ecosystem in Jinyun Mountain.

Seasonal variation

Fig. 3 presents the seasonal variation of major ions in Jinyun Mountain. Among the major ions, the most abundant anion, sulfate ion (SO_4^{2-}), showed pronounced seasonal variation. A distinctive SO_4^{2-} contribution maximum occurred during the winter and dropped to a minimum in the summer because SO_2 emissions were more prevalent in the winter. The two most abundant cations, ammonium (NH_4^+) and calcium (Ca^{2+}), also

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Table 4. *The PCA results for the precipitation of Jinyun Mountain*

ions	components		
	1	2	3
K^+	0.879	-0.207	-0.044
Na^+	0.726	-0.345	0.145
Ca^{2+}	0.852	0.251	0.190
Mg^{2+}	0.762	0.420	-0.067
NH_4^+	0.846	0.298	-0.080
SO_4^{2-}	0.856	0.007	0.377
NO_3^-	0.459	0.738	0.429
Cl^-	0.588	-0.462	0.578
Eigenvalue	4.610	1.249	0.729
percent of trace%	57.6	15.7	9.1

Table 5. *The correlations between ions of precipitation in Jinyun Mountain*

	Na^+	Ca^{2+}	Mg^{2+}	NH_4^+	SO_4^{2-}	NO_3^-	Cl^-
K^+	0.632**	0.775**	0.509**	0.690**	0.738**	0.262*	0.558**
Na^+		0.610**	0.450**	0.466**	0.509**	0.145	0.521**
Ca^{2+}			0.540**	0.617**	0.752**	0.170	0.506**
Mg^{2+}				0.668**	0.649**	0.552**	0.241*
NH_4^+					0.735**	0.541**	0.332*
SO_4^{2-}						0.251*	0.343*
NO_3^-							0.141

**$P<0.01$, *$P<0.05$

showed seasonal variations. NH_3 concentration in the air was much higher in spring and winter and substantially lower in the rest of the seasons because of the agricultural activities in Chongqing. Thus, NH_4^+ had high concentration in spring and winter and low concentration in summer and autumn. On the other hand, Ca^{2+}, an ion mainly introduced from dust, showed the highest concentration in the dry season of winter due to the minimum dilution effect and relatively lower coverage of vegetation.

Discussion and conclusions

Data from the acid rainfall monitoring in Jinyun Mountain from April 1998 to November 1999 were analyzed to characterize the chemical composition of precipitation. Major conclusions were summarized as follows.

The air pollution and acid rain in Jinyun Mountain were relatively less serious compared with those in Chongqing urban areas. The average precipitation pH in Jinyun Mountain was 5.23 due to the neutralization and the average conductivity was 33.90 μs/cm, which showed the air environment in Jinyun Mountain was better than that in Chongqing urban region. But the frequency of acid rain was over 60% and the pollution in winter was severe (the minimum pH was only 3.80 and the average pH was 4.24) in Jinyun Mountain. If no measures are taken to reduce the sulphur emissions, the effects seen today are likely to increase to large areas with impacts on the health of forest ecosystem in the Nature Reserve of Jinyun Mountain. Jinyun Mountain is located in Chongqing suburb, where the precipitation was mainly influenced by the local air pollutants due to the special topography and air environment [12]. But in recent years, the air and precipitation pollution tended to become much severe in Jinyun Mountain. This may be related to two reasons. First, the local air pollutants increased with the

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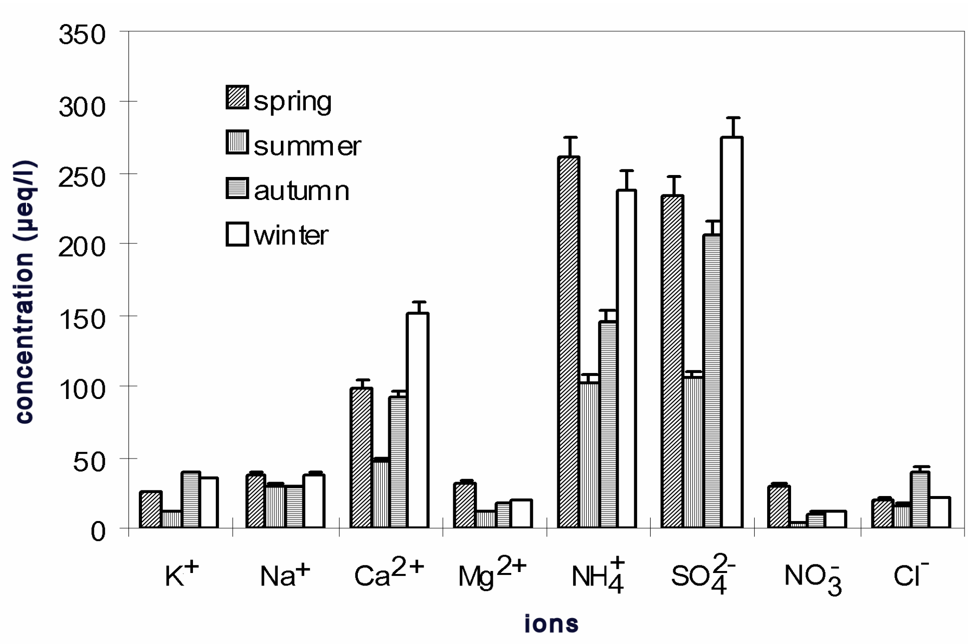

Figure 2. *The seasonal change of precipitation ions in Jinyun Mountain*

development of the regional economy. Second, a part of SO_2 emissions were transferred to Jinyun Mountain from downtown due to high-chimney emission in urban regions. Therefore, in order to decrease the precipitation acidity in Jinyun Mountain and protect the forest ecosystem in the Nature Reserve, it is useful to control the total SO_2 emissions including in the suburb and urban areas of Chongqing. It is also suitable in other cities of China.

The acid rain in Jinyun Mountain was also classified to sulphuric acid type and the concentration of SO_4^{2-} accounted for more than 80% of the total anions. The concentration of anions, especially, SO_4^{2-} was significantly lower than that in Chongqing urban areas, but was still much higher than that in other developed countries in Northeast America, Europe and East Asia and did harm to the health of ecosystem. NH_4^+ and Ca^{2+} were the most abundant cations in Jinyun Mountain precipitation, which accounted for 77.36% of the total cations. Compared with the precipitation in Northern China and Chongqing urban areas, the concentration of Ca^{2+} was much lower while the concentration of NH_4^+ was significantly higher, which were related to the characteristics of local environment in Jinyun Mountain. The chemical composition of precipitation in Jinyun Mountain showed obvious seasonal variation. The air pollution was much severe in winter and more favourable in summer, which may be related to the seasonal variation in precipitation amount.

The analysis of PCA and correlation on major ions of precipitation showed that the abundant NH_4^+ and Ca^{2+} neutralized greatly the acidity of acid ions. Consequently, precipitation with relatively high pH was observed and reduced the direct damage to forest vegetation. But some scientists have found that this neutralized precipitation with weak acid substance [such as $(NH_4)_2SO_4$] is harmful to the ecological environment after it gets into the soil [20]. The NH_4^+ can acidify the soil through the plant absorption and the nitrosation of nitrous bacteria; the movement of sulphate ion can also lead to the soil acidification by improving the loss of base cation. Such precipitation called acidifying precipitation is not acid precipitation, but still can hurt the health of plant communities

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finally by influencing the soil quality. There are no direct negative influences when SO_4^{2-} coexists with K^+, Na^+, Ca^{2+} and Mg^{2+}, but it is still a potential threat to ecosystem [20]. Therefore, it is necessary to control the acid and alkaline sources simultaneously in order to protect the acid and alkaline balance in the ecological environment and reduce the damage caused by air pollutants.

In the terrestrial ecosystem, forest communities have large biomass and usually became the direct victims of the acid deposition due to the interception by the tree canopy. At the same time, the soil quality decreased because of the long-term influence of acid rain and acidifying rain, which also do harm to the plant health. There have been many research works conducted on the influence of acid rain on coniferous forest ecosystem due to its sensitivity [13, 21, 22]. The main vegetation type in Jinyun Mountain is sub-tropical broadleaved evergreen forest that has better ability to absorb acid ions and resist the damage of acid rain compared with coniferous forest [1, 13, 22]. But the number of detailed research works on broadleaved evergreen forests is limited. The broadleaved evergreen forest is the climax vegetation in subtropical regions, the ecological restoration is more difficult than other forest if destroyed. There may be time delays between the onset of acid deposition and any resulting ecosystem impacts; so careful and integrated consideration is required to assess acid and acidifying deposition in broadleaved forest ecosystem.

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SEED GERMINATION AND REPRODUCTIVE STRATEGIES OF TITHONIA DIVERSIFOLIA (HEMSL.) GRAY AND TITHONIA ROTUNDIFOLIA (P.M) BLAKE

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Abstract. Seed germination and reproductive strategies of *Tithonia diversifolia* and *Tithonia rotundifolia*, two invasive species introduced into Africa from North and Central America, were studied. The aim was to determine the characteristics that make them invasive species in the continent. *Tithonia diversifolia* is a perennial and polycarpic plant reproducing both sexually and asexually while *Tithonia rotundifolia* is an annual monocarpic plant reproducing only sexually. The seeds of these species exhibit a period of dormancy before germinating. *Tithonia diversifolia* produces small sized light and numerous seeds while *Tithonia rotundifolia* produces larger sized, heavier and fewer seeds. *Tithornia rotundifolia* allocates a high proportion of of dry matter (41.6 %) and *Tithonia diversifolia* low proportion (10.5 %) to reproduction. It is concluded that small sized light and numerous seeds produced by *Tithonia diversifolia* accounts for its wide dispersal and rapid spread in colonized areas. Also its perennial habit and ability to reproduce sexually and vegetatively accounts for the species colonizing and stabilizing fast in new habitats. While the larger heavier seeds and high reproductive effort of *Tithonia rotundifolia* ensures its early vigorous start in seedling growth, quick establishment, survival for longer and to grow to more aggressive size in an environment that is starved of resources. Seed dormancy exhibited by the two species ensures they survive adverse conditions in their environment as seeds and germinate only when the environmental conditions favour the survival of their seedlings.
Keywords. *Tithonia species, reproductive strategies, seed dormancy.*

Introduction

Environmental weeds are those plants that invade natural vegetation, usually adversely affecting the survival of the native flora. These invasive plants are composed of herbs that may be annual, biennial or perennial as well as perennial vines, shrubs and trees. A large proportion of these invasive plants of terrestrial habitats are species of essentially open, sunny habitats, others are adapted to varying degrees of lower light levels as found under thickets or forest canopies. In general, invasive plants are characterized as being highly adaptable to a broad range of environmental parameters. The adaptability to varying types of substrate, levels of moisture, quality of light and temperature regimes allows them to invade a broad range of ecosystems and habitat types. They tend to be highly successful as judged, in part, by their ability to produce an abundance of seeds, that are in some cases may have a long life in the soil seed bank. Disturbance of natural ecosystems by a wide range of human actions are the primary cause for the successful spread and proliferation of these species. The ultimate result is that ecological conditions are created that promote the establishment and spread of these species that actively compete and replace native species. These invasive plants are introduced deliberately, although accidental introductions occur through arrival of livestock, as contaminants with grain and in ballast and soils. Movement within the country may involve natural dispersal (by wind or water), animal movements (native,

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domestic and feral animals disperse the seeds), vehicles, transport of soils and agricultural products and so on.

The process of individual development represents a strategic allocation of resources to conflicting ends. The concept of allocation depends absolutely on the idea that different structures or activities are alternatives, that a gain in one as a result of selection must be offset by a loss in another. Plants appear to possess only limited resources which are shared between the competing demands of maintenance, growth and reproduction. The fraction of its available resources that a plant devotes to reproduction may vary with environmental and genetic factors and is co-adapted part of the whole process that constitutes a life history [14]. Plant's quantitative programme of resource allocation is an essential feature of its strategy. Life cycle strategy is the whole complex time and space of resource allocation by plants while reproductive strategy describes the resource allocation particularly associated with reproduction [6]. As the size of a seedling represents, both the product of the embryonic capital, the growth rate and the time elapsed since germination, both seed size and dormancy constitute important quantitative features of reproductive strategy [6].

The number of seeds produced by a plant, the number of seeds it fathers with the pollen it produces and the proportion of these offspring which survive to reproductive maturity are factors which determine how many descendants left by a genotype expressing a particular life history pattern [5]. The seed is a dormant or resting stage in the life of a plant and the stage of the life cycle at which dispersal and colonization of new areas occurs. Seeds survive adverse conditions better than growing plants and thus plants "ride out" difficult environmental circumstances in the seed state with low levels of metabolic activity and resume active growth when more favourable conditions return [8]. The survival value of these resting stages in the life cycles of plants has led to the evolution of different types of seed dormancy states, which can persist for a long time and difficult to break [3, 4, 13]. Harper [4] recognized three types of seed dormancy – innate, induced and enforced-which play slightly different roles in the regulation of germination. Innate dormancy is normally due to endogenous factors such as immaturity of embryo or the presence of inhibitors, it can be overcome with a period of after ripening or often by some seasonal stimulus, for example, photoperiod, thermoperiod. Induced dormancy develops in seeds when an adverse factor acts upon the seed and produces a suspended animation that continues after the causal factor has ceased to act. Enforced dormancy is imposed by an exogenous factor (e.g. carbon dioxide narcosis) and lasts only as long as the factor acts upon the seed.

Recently two species, *Tithonia diversifolia* and *Tithonia rotundifolia*, of the 11 species of the genus *Tithonia*, native to North and Central America have been introduced, are naturalized and have become invasive species in Africa. These two species have become naturalized in Southern Africa while *Tithonia diversifolia* has naturalized in West Africa. In these areas, the species have established themselves as serious weeds of arable crops, plantations, abandoned lawns and roadsides. They are aggressive colonizers of new sites, colonizing every available sunny space with high water table. They are alloptric, never found growing in mixed population. Opinions vary as regards their introduction and subsequent establishment. In West Africa, *Tithornia diversifolia* has been reported to be introduced as an ornamental plant [1] and with imported grains [11].

Because of the rate these species are spreading, colonizing every available open space especially along roadsides and displacing the native species in areas where they occur, this study was carried out to investigate the reproductive strategy and seed germination of these *Tithonia* species occurring in Africa with the aim of determining the characteristics responsible for their invasive habits.

Materials and methods

Natural populations of *Tithonia divesifolia* and *Tithonia rotundifolia* at peak growth growing in Lusaka, Zambia were used for this study. In each species population, the plants were tagged by numbering them. Four plants were randomly selected using the number tags. The plants were clipped to ground level. Each plant was separated into leaf, reproductive parts (flowers, fruits, seeds) and stem. These were oven dried at 80 ^{0}C to constant weight and weighed separately. The number of capitula (heads) per plant and seeds per head were counted. One hundred (100) seeds from each plant were oven dried at 80°C to constant weight and weighed. The number of branches per plant was counted for the four plants. The following data were recorded for each individual plant (i) dry weight of reproductive parts, (2) dry weight of leaves, (3) dry weight of stems, (4) number of heads, (5) total number of seeds, and (6) dry weight of 100 seeds. The ratio of dry weight of reproductive parts (heads, flowers, fruits, seeds) to the total dry weight of above ground tissue of all individuals of each species as the index of the fraction of total available resources allocated to reproduction was calculated. This ratio is here defined as 'reproductive effort'.

Mature seeds collected from these plant species on different dates, *Tithonia diversifolia* on 21 June 2004 and *Tithonia rotundifolia* on 30 April 2004 were subjected to germination experiments immediately. They were subjected to scarification with concentrated sulphuric acid in an attempt to break seed dormancy. Standard germination test was carried out by placing seeds taken from the flower heads on moist filter paper lined in 7-cm diameter petri-dish. Each trial had four replicates, each of twenty-five seeds. The test was carried out at ambient temperature for 30 days. Each petri-dish was placed on a bench near a window and watered regularly. Germination was recorded when the radicle emerged.

Scarification with concentrated sulphuric acid was carried out by immersing and shaking 100 seeds each in 100 ml of acid in 500 ml-conical flasks for 4, 6, 10, 15, 20, 25, 30 minutes. The seeds were rinsed thoroughly with distilled water and 25 seeds each placed in petri-dishes (4 replicates of every treatment) lined with filter papers. They were regularly watered with distilled water. Each petri-dish was placed on a bench near a window. Germination was recorded when the radicle emerged.

In all cases seeds were examined every day for 30 days and all germinated seeds were counted and removed from the dishes. Germination was expressed as percentage.

Results

Reproductive tissue production of Tithonia diversifolia and Tithonia rotundifolia.

Tithonia diversifolia is a perennial and polycarpic plant that flowers in late April/May while Tithonia rotundifolia is an annual monocarpic plant flowering in February and completes its life cycle –flowering, setting seeds and dying by the end of growing season in Zambia. It survives as seed. Tithonia rotundifolia reproduces from only seeds while Tithonia diversifolia reproduces from seeds and vegetative regrowth of basal stem when the plant is slashed. Tithonia diversifolia produces higher number of

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capitula and seeds per plant than *Tithonia rotundifolia* (Table 1). It also has higher number of number of seeds per capitulum than *Tithonia rotundifolia* (Table 1). The seeds of *Tithonia diversifolia* are smaller in size, lighter in weight and more numerous than those of *Tithonia rotundifolia* which are larger in size, heavier in weight and fewer in number (Table 1). Thus *Tithonia diversifolia* produces smaller sized lighter and more numerous seeds than *Tithornia rotundifolia*.

Table 1. *Summary of reproductive tissues production at peak growth of Tithonia diversifolia and Tithonia rotundifolia. Values are means and ±95 % confidence interval.*

Reproductive structure	Species	
	Tithonia diversifolia	**Tithonia rotundifolia**
Number of capitula per plant	755.25±286.24	128.00±12.72
Number of seeds per capitulum	179.75±2.32	133.33±5.72
Number of seeds per plant	134,451.75±49,792.14	17629.33±3843.23
Dry weight of hundred seeds (g)	0.53	1.24

Allocation of dry matter to different structures and reproductive effort of the species.

Tithonia diversifolia is shrubby branching profusely from the base without any distinguishable main stem. The mean number of branches per plant is 29±6 branches. *Tithonia rotundifolia* has a distinguishable main stem which branches a little above the ground. The mean number of branches per plant is 21.5±1.4 branches.

Tithonia diversifolia allocated highest amount of dry matter to stem production and lowest amount to leaf production while *Tithonia rotundifolia* allocated highest amount of dry matter to reproductive tissues (heads, fruits, seeds, flowers) production and the lowest amount to leaf production (Table 2).

Table 2. *Summary of allocation of dry weight (g per plant) to different structures and reproductive effort at peak growth of Tithonia diversifolia and Tithonia rotundifolia. Values are means ±95 % confidence interval.*

Structure	Species	
	Tithonia diversifolia	**Tithonia rotundifolia**
Leaf	553.84±215.86	46.89±7.74
Reproductive tissue	671.35±223.86	106.46±36.37
Stem	5146.86±1393.99	102.56±25.25
Total above-ground tissue	6372.05±1752.93	255.91±52.47
Reproductive effort	0.105	0.416

Ratio of dry weight of reproductive tissue to total dry weight of above –ground tissue adapted as the index of the fraction of total available resources allocated to reproduction (reproductive effort) in this study is 0.105 for *T. diversifolia* and 0.416 for *T. rotundifolia* (Table 2). This implies that *T. diversifolia* allocated 10.5 % and *T. rotundifolia* 41.6 % of the available resources to reproduction.

Seed germination and dormancy of these species.

Mature *Tithonia rotundifolia* seeds collected on 30 April 2004 and subjected to germination test in distilled water for four months (May-August) did not germinate (0 % germination). When treated with sulphuric acid for 4 and 6 minutes in July and August the seeds still did not germinate. On treatment with the same acid for 10 minutes in August 2004, 12 per cent germination was recorded. However, in September and October 2004, germination test in distilled water gave 30.7 to 45 per cent germination. Scarification in concentrated sulphuric acid for 15, 20, 25, and 30 minutes in September and October 2004 gave 16, 30, 41.3 and 40.2 per cent germination respectively.

Seeds of *Tithonia diversifolia* collected in June 2004 when they were matured and subjected to germination in distilled water in June 2004 gave 16.3 per cent germination. When subjected to sulphuric acid treatment for 4, 6, and 10 minutes in July and August 2004 gave 28, 40 and 62.7 per cent germination respectively. However, germination tests in distilled water in September 2004 gave 97.5 per cent and after acid treatment for 15 and 20 minutes gave 55 and 73.3 per cent germination respectively.

However, when these seeds were planted in the field in December 2004 during the rainy season, the percentage germination was over 75 per cent for each of the two species.

The above results indicate that the seeds of these two species experience a kind and period of dormancy before germination.

Discussion

Tithonia diversifolia and *Tithonia rotundifolia* whose reproductive strategies and seed germination were investigated in this study are invasive plants introduced to Africa by humans which have become established and spread into natural ecosystems. They have also become serious weeds of arable crops and plantations in Africa. *Tithonia diversifolia* is fast colonizing fallow lands especially abandoned lawns and roadsides in the forest regions of West Africa as *Chromolaena odorata*, another introduced species into the region had done. Similar fast spreading (invasive) habit of these two species in Zambia, a southern African country where this study was carried out has been observed. The lasting and pervasive threat to natural and agricultural ecosystems in Africa by biological invasion of these species is underestimated and ignored. Their introduction into the continent has probably stopped but the species have continued to spread, naturalize and stabilize in the continent and are probably adversely affecting the composition of both native flora and fauna and altering ecosystem processes of the region.

This study shows that *Tithonia diversifolia* produces smaller sized, light weight and larger number of seeds than *Tithonia rotundifolia*. This implies that *T. diversifolia* seeds are generally likely to be more widely dispersed and have a potential of rapid colonization of sites. So that in an environment that is open and colonizable, the seeds will be at an advantage. This probably accounts for the rapid spread of *T. diversifolia*. Also, *T. diversifolia* perennial habit and the ability to reproduce sexually and vegetatively may account for the species colonizing new habitats and stabilizing fast in colonized sites. The plant coppices profusely when the stem is cut. Vegetative reproduction allows it to occupy a temporary site quickly while light seeds produced by sexual reproduction allow distance dispersal to new sites. Reported allelopathic effects [2, 15], such as secretion of compounds from the roots or leaching from the leaves that

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inhibit the seeds of other plants from germinating in the immediate vicinity of the plant together with dense shading may be the reason why few if any plants grow under the canopy. This is probably why *T. diversifolia* stabilizes as single or near single species stands wherever it is found growing.

The large heavier seeds of *T. rotundifolia* implies that its embryo is large and that it carries large food reserves. The large embryo and large food reserves of the seed make it possible for the seedlings to emerge as a more completely developed plantlet, survive for longer and grow to a more aggressive size in an environment that is starved of resources. Johnson and Cook [9] have reported that since major portion of the weight of a seed is food storage tissue, one may conclude that there is some optimum amount of food reserve which will normally insure the necessary seedling vigor. This probably accounts for the early vigorous start in seedling growth and the species quick establishment in an environment. The larger heavier seeds may restrict its dispersal and probably accounts for the species not being as widely spread as *T. diversifolia* though the seed dispersal of these species was not investigated in this study.

The difference in seed size and number observed in these species with *T. diversifolia* producing more seeds per plant which are smaller in size than those of *T. rotundifolia* agrees with the assertion that size (or more strictly the weight) of each propagule and the number of propagules produced per plant are normally somewhat complementary since the total amount of photosynthates available for the production of propagules is limited in each species [7, 10]. Thus, if the number of propagules produced per plant increases, the size of each propagule inevitably decreases or vice versa [16, 17].

Tithonia rotundifolia at peak growth was found to allocate more dry matter to total reproductive structures than *T. diversifolia* in this study. Hickman [7] has reported that the proportion of dry matter allocated to reproductive organs was increasingly great in successively harsher and more open habitats. Also, MacArthur and Wilson [12] reported that reproductive allocation should be highest in areas where available resources are least fully utilized and cite fugitive species as examples. In such habitats they concluded there is an evolutionary premium on filling the available resource space with offspring as rapidly as possible. Such species must have high reproductive allocation both to utilize the available resources more rapidly than other fugitive species and also ensure that propagules find new temporary environments effectively. In this study *T. rotundifolia* was found to allocate as high as 41.6 % dry matter to reproductive structures. This high reproductive allocation and fast growth ensures that *T. rotundifolia* invades new sites and utilizes available resources in such habitats. In an on going experiment, *T. rotundifolia* seeds planted on 10 December 2004 flowered on 14 February 2005; two months and one week after planting while *T. diversifolia* planted at the same time are still young plants.

Seeds of these species displayed a kind of dormancy. *Tithonia diversifolia* showed low germination (16.3 %) immediately after seed was harvested from the field but steadily increased until 4 months after harvest when 97.5 per cent germination was obtained. But the seeds of *T. rotundifolia* did not germinate immediately until 4 months (30 per cent germination) after harvest from the field. The highest germination percentage was 45 per cent after 5 months. This period of initial dormancy was completed when the seeds were stored dry at room temperature. Scarification of these seeds with concentrated sulphuric acid for varying periods did not improve the germination of these seeds especially those of *T. rotundifolia*. Thus, it is concluded that that the seeds of these species exhibit either innate dormancy due to immaturity of the embryos which required some period of after ripening to reach maturity or enforced dormancy due to low temperatures prevalent in Zambia between April and August. This dormancy was broken probably by seasonal stimulus specifically thermoperiod because of higher temperatures in Zambia between September and November. Seed dormancy enables plants survive adverse environmental conditions with low levels of metabolic activity and to resume active growth when more favourable conditions return. It also allows a timing of germination in a periodically fluctuating environment. Seed dormancy observed in these species ensures that they survive adverse conditions in their environment as dormant seeds only to germinate when the environmental conditions favour the survival of their seedlings.

It is concluded from this study that some of the characteristics contributing to invasive habits of *Tithonia diversifolia* and *Tithonia rotundifolia* are seed dormancy in both species, small sized light and numerous seeds production and sexual and vegetative reproduction of *T. diversifolia* and large sized seeds and high reproductive allocation of *T. rotundifolia*. Reproductive allocation was determined here using single harvest at peak growth (maturity) which Hickman [7] reported is more satisfactory than multiple harvests (at different points in the growth cycle of plants) which are difficult to integrate. The only limitation of this method was that shed plant parts during the growth cycle of these species were not determined and used in the calculation of reproductive allocation in this study.

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EFFECTS OF SOIL TREATMENTS CONTAINING POULTRY MANURE ON CRUDE OIL DEGRADATION IN A SANDY LOAM SOIL

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Abstract. The impacts of crude oil pollution on a sandy loam soil and the influence of incorporation of poultry manure alone and in combination with alternate carbon substrates (glucose or starch) and surfactants (Goldcrew or Corexit) were investigated. Oil pollution increased soil organic carbon and reduced soil nitrates and phosphorus, thus imposing a condition that impaired oil degradation in the soil. Treatment of the soil with poultry manure alone, enhanced oil degradation but the extent of this was influenced by the incorporation of alternate carbon substrates or surfactants. Addition of glucose or Corexit encouraged crude oil degradation, while addition of starch or Goldcrew reduced the extent of degradation obtained. The soil amendments interacted in affecting crude oil degradation. This was optimal with a combination of poultry manure (2.0%w/w) + glucose (2.0%w/w) which yielded a crude oil degradation of 7.42±1.02% after sixteen weeks incubation.
Keywords. *alternate carbon substrates, crude oil degradation, poultry manure, surfactants*

Introduction

Crude oil pollution adversely affects the soil ecosystem through adsorption to soil particles, provision of an excess carbon that might be unavailable for microbial use and induction of a limitation in soil nitrogen and phosphorus [3, 4]. These cause a delay in the natural rehabilitation of crude oil polluted soils and various soil treatments have been used in bioremediation strategies to hasten the process. These include surfactants, alternate carbon substrates and organic and inorganic nitrogen and phosphorus. The effectiveness of these treatments has however been conflicting [8, 10, 15]. This might be related to the heterogeneity of soils and crude oil samples as well as possible interactions between the soil amendments and the natural soil constituents [14]. The effectiveness of each treatment in any soil therefore needs to be evaluated on a case specific basis.

Poultry manure has over time been used to improve soil fertility [23]. Its efficacy in promoting plant growth in crude oil polluted Nigerian soils has also been reported [1, 21]. This study investigates the impact of addition of poultry manure alone and in combination with surfactants or alternate carbon substrates to enhance crude oil degradation in a sandy loam soil.

Materials and methods

Samples and sample collection

Soil sample was collected randomly with a Dutch auger at a depth of 15 cm from an agricultural farm in Port-Harcourt, Nigeria. Samples were homogenized, dried, sieved through a 2 mm mesh and stored in polythene bags at room temperature (28±2 °C) in the laboratory. The crude oil was a Nigerian Bonny medium blend obtained from Shell Petroleum Development Company (SPDC) Limited, Port-Harcourt, Nigeria.

Soil amendment materials included NPK (20:10:10) fertiliser obtained from National Fertilizer Company (NAFCON), Port Harcourt, Nigeria. Goldcrew and Corexit surfactants were obtained from SPDC. The poultry manure was obtained from a poultry farm in Port Harcourt. It was air dried, crushed and stored in the laboratory at room temperature (28±2°C) before use.

Soil characterization

The soil was characterized before pollution and two weeks after pollution with crude oil. Particle size determination was done by the hydrometer method [5]; pH was determined according to the modified method of McLean [16]; total organic carbon was determined by the modified [19] wet combustion method [26] and total nitrogen was determined by the semi-micro Kjeldhal method [7]. Available phosphorous was determined by Brays No.1 method [22]. The exchangeable cations, sodium and potassium were determined by flame photometry. Ammonium-nitrogen was determined by the nesslerisation method [13] while nitrate-nitrogen was by the phenoldisulphonic acid method [6].

Soil microbial population was estimated by the ten-fold serial dilution method [11]. Population of total heterotrophic bacteria and fungi were estimated using nutrient agar (Oxoid) and potato dextrose agar respectively. Populations of petroleum hydrocarbon utilising bacteria and fungi were estimated using the vapour phase transfer method [2] a mineral salt medium [12].

Pollution and amendment of samples

Twenty gram soil portions weighed into 100 ml bottles were moistened to 60% of their field moisture capacity and left at room temperature (28±2°C) in the laboratory for one week. Thereafter the samples were treated with 10%v/w crude oil and left at the same temperature for another two weeks. A basal dressing of NPK (20:10:10) fertilizer was applied at a concentration of 1250 μg/g soil. The effects of the various soil amendments were studied as in *Tables 2* and *3*, and *Fig. 1*.

In each study, two control units of the soil were also set up. The polluted control was treated with 10%v/w crude oil and NPK fertilizer while the unpolluted control was treated with only NPK fertilizer. Both the amended soils and the controls were incubated at room temperature (28±2°C) in the laboratory for four weeks. Thereafter the soils were air-dried, homogenized and oil content estimated. Changes in oil content in the treatments were calculated relative to the oil content in the polluted and un-amended control.

Crude oil degradation and carbon dioxide production in amended soils

This study investigates the extent of crude oil degradation and carbon dioxide production obtained with time using the soil treatment that gave optimal crude oil degradations in the previous studies (poultry manure + glucose at 2.0%w/w + 2.0%w/w. Twenty gram soil portions weighed into 100 ml bottles were moistened to 60% of their field moisture capacity and left at room temperature (28±2 °C) in the laboratory for two weeks. Thereafter the samples were treated with 10%v/w crude oil and left at the same temperature for another four weeks. A basal dressing of NPK (20:10:10) fertilizer was applied at a concentration of 1250 μg/g soil. The samples were then variously treated

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Table 1: *Soil properties before oil pollution and two weeks after oil pollution*

chemical	soil properties	
	before oil pollution (mean±SEM)	two weeks after oil pollution (mean±SEM)
pH	6.10±0.10[a]	4.90±0.01[b]
organic C(%)	2.14±0.02[b]	7.06±0.06[a]
total N(%)	0.15±0.00[a]	0.18±0.01[a]
C : N ratio	14.27	39.22
nitrate N (ppm)	55.35±0.35[a]	12.30±0.05[b]
ammonium N (ppm)	5.93±0.01[b]	7.22±0.01[a]
available P (ppm)	20.00±0.50[a]	10.88±0.01[b]
Exchangeable cations (meq/100g)		
Na	0.17±0.01[b]	6.08±0.01[a]
K	0.78±0.01[a]	1.28±0.01[a]
Microbiological		
Bacterial populations ($\times 10^8$ $cfug^{-1}$soil)		
total heterotrophs	1.88±0.14[b]	4.00±0.30[a]
petroleum hydrocarbon utilisers	0.76±0.13[a]	0.85±0.05[a]
Fungal populations ($\times 10^5$ $cfug^{-1}$soil)		
total heterotrophs	0.72±0.08[b]	1.72±0.08[a]
petroleum hydrocarbon utilisers	0.41±0.06[b]	1.63±0.19[a]

Within row, mean±SEM with different superscripts are significantly different at P<0.05

with the soil amendments at the concentrations previously found optimal for crude oil degradation (*Fig. 2*). Polluted and unpolluted controls were also set up and the samples incubated as previously described. Replicate samples were analysed at 0, 2, 6, 9, 12 and 16 weeks intervals and changes in oil content calculated relative to the oil content in the polluted and un-amended control. Samples for carbon dioxide production were similarly treated and set up in 250 ml screw-capped bottles.

Determination of oil content

Oil content was determined spectrophotometrically according to the toluene extraction method [20]. One gram (1 g) of air-dried and homogenized soil was weighed into 50 ml conical flasks and ten millilitres of toluene (solvent) added to extract the oil in the soil. After shaking vigorously, the mixture was allowed to stand for 10 minutes and then it was filtered through Whatman No. 1 filter paper. The extracted oil was diluted appropriately with fresh toluene and the absorbance read at 420 nm in Spectronic 21 spectrophotometer.

Determination of carbon dioxide evolution

Carbon dioxide production was determined and calculated according to the methods of Cornfield [9] and Stotzky [25]. To absorb the carbon dioxide liberated during oil degradation, vials containing 10%w/v of barium peroxide in distilled water were placed inside the 250 ml screw-capped bottles containing the soil treatments. The vials were withdrawn after four weeks during the pollution and amendment studies (section 2.3) and at 0, 2, 6, 9, 12 and 16 weeks intervals during the degradation studies (section 2.4). The amount of the carbon dioxide absorbed was determined by titrating the barium carbonate formed with 1 N hydrochloric acid.

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Analysis of findings

Each experiment was carried out in triplicates. Data collected were subjected to analysis of variance (ANOVA) and the Duncan's multiple range tests (DMRT) [24]. The relationships between the variables were established using the correlation analysis.

Results and discussion

Pollution of the sandy loam soil by crude oil led to an increase in soil organic carbon from 2.74±0.02% to 7.06±0.06% as well as in the populations of total heterotrophic microorganisms (*Table 1*). There was however a reduction in nitrate-nitrogen and available phosphorus from 55.35±0.35 ppm to 12.30±0.05 ppm and 20.00±0.50 ppm to 10.88±0.01 ppm respectively. Crude oil pollution therefore adversely affected the soil properties. The increased microbial population – despite this – represented an immediate

Table 2: *Effects of soil treatments containing poultry manure on crude oil degradation.*

soil treatment*	oil content (ppm) (mean±SEM)	change in oil content (ppm) (mean±SEM)	% change in oil content (mean±SEM)	CO_2 production (mg / 20 g soil) (mean±SEM)
poultry manure	57014.36±389.39^{c}	-2659.53±94.69^{b}	-4.46±0.34^{b}	28.6±1.0^{c}
poultry manure +Goldcrew	58863.96±194.69^{bc}	-809.93±0.00^{d}	-1.36±0.01^{d}	33.0±1.0^{b}
poultry manure +Corexit	55649.63±292.04^{d}	-4024.26±486.74^{ab}	-6.74±0.78^{ab}	24.2±0.2^{d}
poultry manure +glucose	55064.98±194.69^{d}	-4608.91±0.00^{a}	-7.72±0.05^{a}	24.2±0.2^{d}
poultry manure +starch	61876.98±11362.86^{a}	+2203.09 ±1168.17^{f}	+3.69±1.95^{f}	8.8±1.0^{e}
poultry manure +Goldcrew +glucose	58181.31±194.69^{bc}	-1492.58±0.00^{c}	-2.50±0.01^{c}	52.8±1.0^{a}
poultry manure +Goldcrew +starch	57009.26±48.67^{c}	-2664.63±146.02^{b}	-4.47±0.22^{b}	26.4±1.0^{cd}
poultry manure + Corexit + glucose	57889.27±584.08^{bc}	-1784.62±389.39^{c}	-2.99±0.67^{c}	30.8±1.0^{b}
poultry manure + Corexit + starch	55938.79±778.78^{d}	-3735.10±584.39^{ab}	-6.23±1.01^{ab}	55.0±0.5^{a}
control (Un-amended)	59673.89±194.69^{b}	0.00±0.00^{e}	0.00±0.00^{e}	22.0±0.5^{de}

Within column, mean±SEM with different superscripts are significantly different at P<0.05.
**Concentrations of soil treatments: Poultry manure: 1.0%w/w; Goldcrew and Corexit (surfactants): 0.01% v/w; glucose and starch (alternate carbon substrates): 0.5%w/w*

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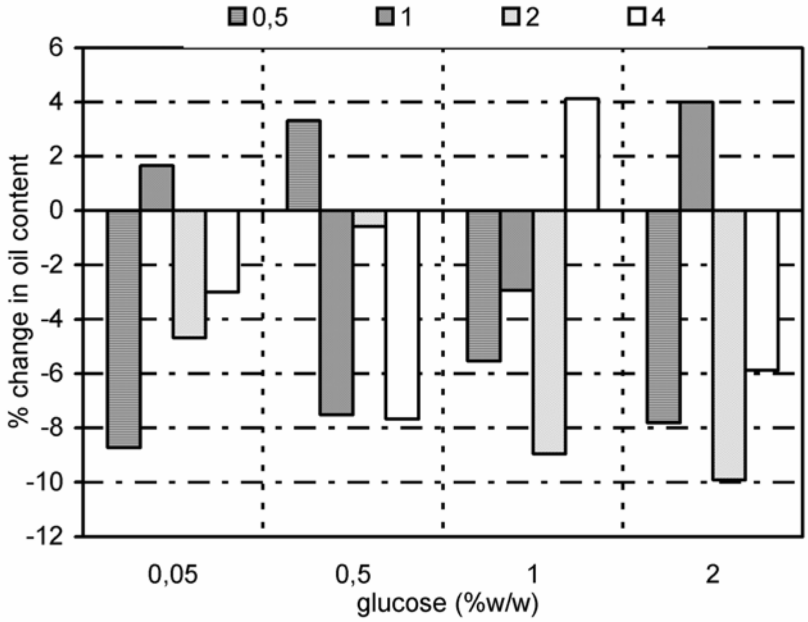

Figure 1. *Effects of different concentrations of glucose and poultry manure on crude oil degradation.*

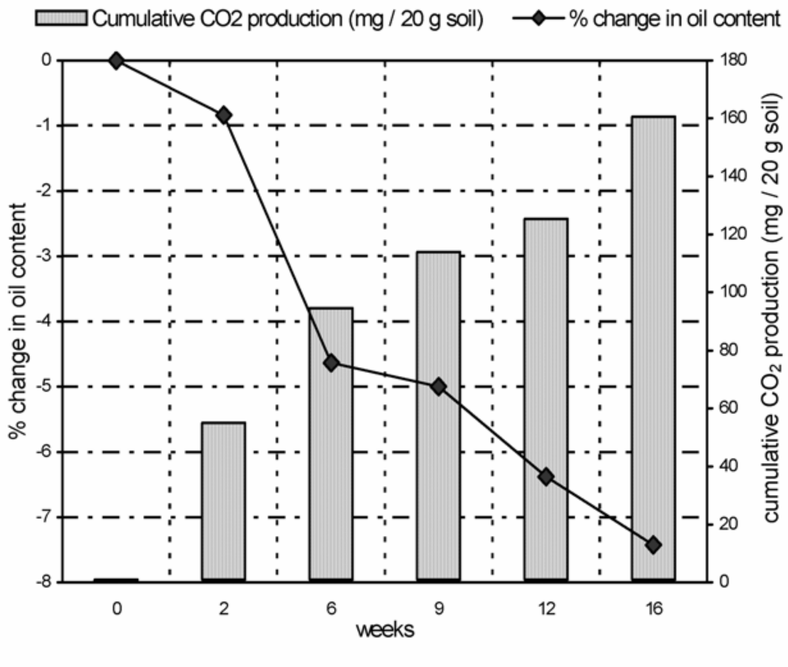

Figure 2. *Crude oil degradation and cumulative carbon dioxide production in soil treated with poultry manure (2%w/w) + glucose (2%w/w)*

response to the added organic carbon, which provided an additional carbon substrate for microbial growth and multiplication. This increase is however transient since these microbes will utilize the already depleted soil nitrogen and phosphorus which eventually will become limiting and cause a reduction in microbial population with time [17].

The results of the effects of the addition of poultry manure alone and in combination with surfactants and/or alternate carbon substrates are presented in *Table 2*. Apart from the sample treated with poultry manure + starch in which a reduction in crude oil.

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degradation relative to the un-amended control was observed, enhanced crude oil degradations were obtained from all the other treatments. The enhanced oil degradations were obtained from all the other treatments. The enhanced oil degradation observed with the addition of either of the surfactants to poultry manure + starch mixture indicate that the soil amendments interacted in enhancing crude oil degradation. Maximum oil reduction of 7.72±0.05% relative to the un-amended control was observed from sample treated with poultry manure + glucose. A significant ($P<0.05$) positive correlation ($r = 0.59$) existed between carbon dioxide production and extent of crude oil degradation. Glucose must have provided a more bio-available alternate carbon substrate that ensured a vibrant microbial population that led to the increased crude oil degradation. This is contrary to the observation that in the presence of a more readily degradable carbon substrate, microbes will prefer the carbon source that needs less energy to degrade [18]. It has been observed that microbial mineralisation of organic matter added to soil depends on the interaction between the chemicals in the soil [14]. In this case, it is therefore possible that an interaction between poultry manure, glucose and the soil constituents provided a soil condition most suitable for crude oil degradation in the sandy loam soil.

Results presented in *Fig. 1* showed that this interaction was optimal at a poultry manure + glucose concentration of 2.0%w/w + 2.0%w/w where the greatest crude oil degradation of 9.91±1.17% was obtained. The result of the extent of crude oil degradation observed with time in the sandy loam soil treated with the optimal concentration of poultry manure + glucose is presented in *Fig. 2*. There was a consistent reduction in oil content with time. After sixteen weeks incubation, under the prevailing natural environmental condition, a crude oil degradation of 7.42±1.02% relative to the un-amended control was obtained while the cumulative amount of carbon dioxide produced was 160.0±1.0mg / 20g soil.

Conclusion

Poultry manure enhanced crude oil degradation in the sandy loam soil. The extent of this depended on the presence of other soil amendments. Maximum degradation was achieved with the addition of a mixture of poultry manure and glucose at optimal levels.

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PHOSPHATASE ACTIVITY OF NON-HAIR FORMING CYANOBACTERIUM *RIVULARIA* AND ITS ROLE IN PHOSPHORUS DYNAMICS IN DEEPWATER RICE-FIELDS

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Abstract. Phosphatase activity of *Rivularia* strains II and I isolated from a deepwater rice field was studied. When grown under conditions of P-limitation the strains did not form hair and showed great variations in induction time and pattern of enzyme activity in relation to growth. Phosphomonoesterase activity (PMEase) was induced earlier in the *Rivularia* strain I which was maintained in high phosphate concentration in the medium before the study. *Rivularia* strain II on the other hand showed induction of PMEase activity after 3-4 days and this strain was maintained in low phosphorus concentrations in the medium before the study. The comparison of K_m and V_{max} values early in the growth phase showed that affinity of both PMEase (phosphomonester) and PDEase (phosphodiester) for the substrate was higher in early growth phase rather than in later stages suggesting that there is presence of more than one phosphatase enzymes in these organisms. The observations from the above study have important implications in rice fields where constant fluctuations in nutrients can be observed – especially by phosphorus in its most available form phosphate. Equal enzyme activity in both light and dark for the two strains (non hair-forming) implies that phosphatase activity may not be affected by changes in light regimes in rice fields which do occur due to the plant growth. This can in turn contribute significantly to maintain the growth of cyanobacteria for prolonged periods even under unfavorable conditions of phosphorus depletions. These strains of *Rivularia* can therefore biotechnologically exploited as bio-fertilizers (phosphatic fertilizer) when there is phosphorus deficiency. During the unfavorable conditions of phosphorus deficiency, phosphatase activity may play a significant role in providing phosphate for its own growth and for the rice plants.
Key words. *PMEase, PDEase,* K_m, V_{max}.

Introduction

Phosphatases are enzymes, which promote the degradation of complex phosphorus compounds into orthophosphate and an organic moiety, are thus believed to have an essential function in the nutrient dynamics of most of the ecological niches [11]. Microorganisms with phosphatase activity are able to hydrolyze phosphate from a variety of organic phosphorus compounds [2, 3]. Many cyanobacteria are therefore particularly amenable to studies on phosphatase activity, because they can be studied both as field samples and as laboratory isolates. Phosphatase activity has been found in all major groups and numerous species of algae and is found widespread among P-limited cyanobacteria though not universally [9, 12, 17, 18]. Rice fields constitute a very interesting habitat for the study of phosphatase activity as they are dominated by cyanobacteria and contribute significantly to maintain soil fertility [1, 5, 15]. There is evidence that cyanobacteria belonging to Rivulariaceae occur in environments where organic phosphate is an important source of phosphate [10]. Cyanobacteria with the ability to form hairs occur in environments where organic phosphates are especially important [16]. As the strains showed the tapering characteristics of *Rivularia* under conditions of P-limitations and these strains studied did not form hairs, it seemed important to determine how it differed from hair forming strains.

The aim of the present investigation was therefore to study the changes in (PMEase and PDEase) activity and induction during the different stages of growth of the two *Rivularia* strains II and I isolated from the rice fields of Uttar Pradesh and West Bengal (India). In particular variations in K_m and V_{max} values of substrate concentration curves using the Michaelis–Menten kinetics denoting the substrate affinity relationships at early and later stages of growth of cyanobacteria was studied. Another attempt has been the comparison between the strain II and I subjected to high and low concentrations of phosphate before the study respectively.

Materials and methods

Isolation and purification of algal samples

Rivularia strain I was isolated from the rice fields of Uttar Pradesh, India, identified by taxonomic keys [6, 7, 14] purified and maintained in culture collections of Laboratory of Algal Biotechnology, Department of Bioscience Barkatullah University, Bhopal, India, where it had been subjected to high phosphate concentration and stored in liquid nitrogen, while strain II was isolated from the rice fields of West Bengal (India) and was subjected to low phosphate concentration before the experimentation. The cyanobacterial filaments were microscopically examined, which revealed these strains did not form hairs in culture and they contained scytonemin pigment in abundance.

Culture conditions

Cultures were grown at 30 °C and 100 μmol photon m^{-2} s^{-1} PAR continuous light regime using cool florescent source. The media used was CHU 10 (CHU 10-D) medium as given by [4] and slightly modified by [8] with P reduced to 1 mg^{-1}, EDTA as chelator and pH buffered to 7.6 with HEPES [12]. Materials for inoculations for phosphatase activity and growth were sub cultured twice at 4 days interval, centrifuged inoculated in fresh medium to give 10 mg^{-1} dry weight. Three replicates were used and the flasks were pooled at the time of harvesting to provide sufficient material for measurements of dry weight. The organisms were dried at 105 °C and the yield was recorded as dry weight.

Phosphatase assay

Cyanobacterial cells were harvested every day till induction and there after every two days during growth follow till 20 days for the two strains to monitor changes in phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activity. Cellular material was obtained by centrifugation at 5000 g for 10 minutes.

PMEase activity was assayed routinely using colorimetric method using para-nitro-phenyl-phosphate (p-NPP) and PDEase activity using bis-para-nitro-phenyl-phosphate (bis-p-NPP). The assay was studied by the method given by [19]. As the two strains II and I were subjected to high and low phosphate concentration before the study, cyanobacterial cells were transferred to P-minus medium before the phosphatase assay for two weeks to deplete the cells of P. Assays were carried out in a P free version of the medium and buffered with 100 μM glycine (final concentration) to give a pH of 9. For each replicate, a bottle was filled with definite amount of cold sterile medium and

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algal pellets obtained after centrifugation were added to each bottle excluding the control set. Assays were conducted in a water bath with gentle shaking at 30 °C and light flux (100 μmol photon m^{-2} s^{-1} PAR). Dark conditions were also tested and were obtained by wrapping the bottle twice with aluminum foil. Practical details were given in [13, 19]. Substrate was added to the bottles and bottles were tightly capped and run for 30 min. After the assays supernatant was passed through a GF/C filter (Whattman) and the activity ended using the 10% v/v correct base/acid terminator. For the spectrophotometeric analysis, the absorbance of p-NP and bis p-NP was measured by recording the optical density in a Systronic spectrophotometer Model-169 at 405 nm (nanometers). The results are expressed in terms of product (p-NP, bis-p-NP) formed g^{-1} d wt h^{-1} (p-NP = para-nitro-phenol, bis-p-NP = bis-para-nitro-phenol). The filter containing algal material was dried overnight at 105 °C. The mass of the filter, biomass was recorded and biomass of the algae was determined after correction for the mass of the filter. The yield was expressed in terms of dry weight mg l^{-1}.

Results

Fig. 1 shows the induction of PMEase activity using p-NPP was detectable on day 2, while PDEase activity on the other hand was induced on the 2nd day with negligible amount in strain I.

Induction of PMEase activity was detectable on the 4th day in strain II whereas PDEase activity on the 2nd day (*Fig. 2*).

The PMEase and PDEase activity of strain I increased in the initial stages of growth reaching a maximum on days 10 and 8, respectively, and decreased during the later stages of growth. In strain II both monoesterase and diesterase activity increased with the growth of the alga while decreased during the later stages of growth i.e. around day 28, while in strain I the PMEase and the PDEase activity was induced on day 2 of growth and declined slightly around day 20. In general in the two strains phosphomonoesterase activity was more than phosphodiesterase activity. *Fig. 3* shows the yield in terms of dry weight of strain II and I, during the initial growth of the organism PMEase and PDEase activity was found to be maximum while the activity tends to decrease at the later stages of growth.

Table 1 shows the $V_{\max}$ and K_{m} value of the two strains using p-NPP as monoesterase substrate and bis-p-NPP as a diesterase substrate on days 5 and 15 of growth.

A general observation was that for monoesterase, the affinity of phosphatase enzymes for two substrates was higher on days 5 than on day 15. A study to see whether dark condition had any effect on phosphatase activity reveal that the activity in light and dark was the same when assayed on days 8 and 16 with both p-NPP and bis-p-NPP (*Table 2)*.

Discussion

There were three distinct phases with respect to phosphatase activities during growth of Rivularia in batch culture at optimum light flux. During the first phase (to day 4) there were no cell bound or extracellular activity, this coincides with the period when trichomes of sufficient length appear capable of forming hormogonia.

The second phase (days 5–15) is one of rapidly increasing phosphatase activities, which coincides with the gradual loss of ability to form hormogonia. The third phase

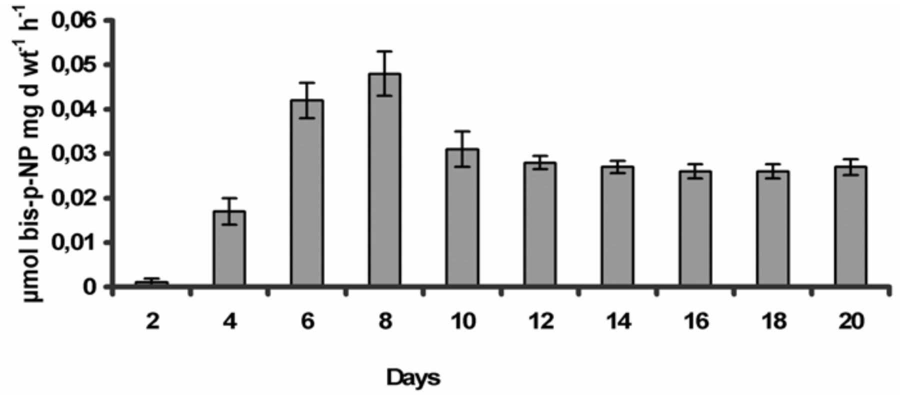

Figure 1. *Phosphomonoesterase (µmol p-NP mg d wt^{-1} h^{-1}) and phophodiesterase (µmol bis-p-NP mg d wt^{-1} h^{-1}) activity in Rivularia strain I.*

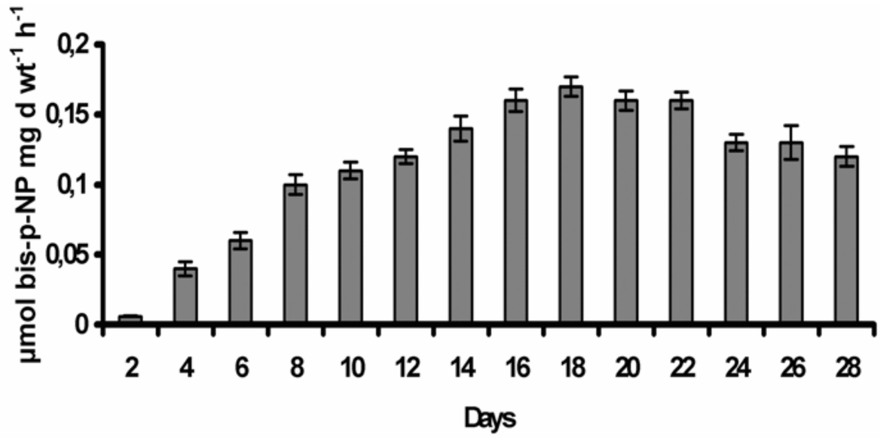

Figure 2. *Phosphomonoesterase (µmol p-NP mg d wt^{-1} h^{-1}) and PDEase (µmol bis-p-NP mg d wt^{-1} h^{-1}) activity in Rivularia strain II over the growth period.*

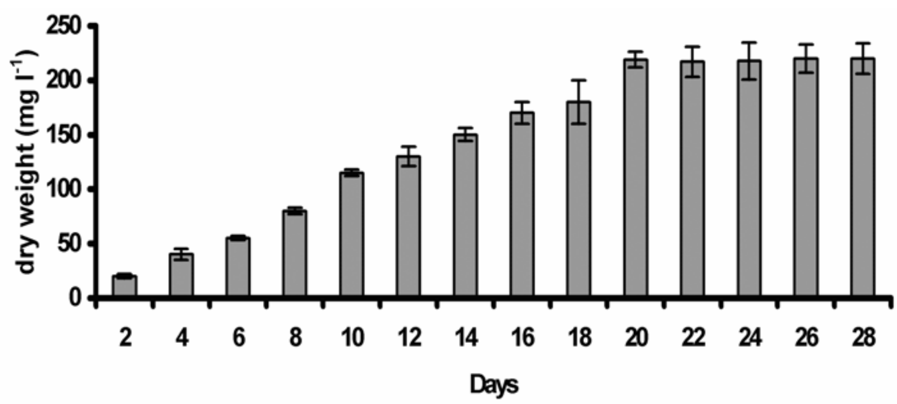

Figure 3. *Graph showing yield in terms of dry weight (mg l^{-1}) in Rivularia strains I and II.*

(days 15–26) is one where activity (PMEase and PDEase) decreases this could be due to the fact that phosphate was removed from the medium within the first few days and stored by the trichomes which had already formed, hormogonia released subsequently were exposed to an environment low in phosphate.

Table 1. *V_{max} (μ mol product m g^{-1} h^{-1}) / K_m (μM) values of Rivularia strain II and I on days 5 and 15 (μmol product mg^{-1} h^{-1} / μM).*

day	substrate	cyanobacterial strains I	II
day 5	p-NPP	0.21 / 11.21	0.13 / 12.98
	bis-p-NPP	0.019 / 10.76	0.08 / 37.86
day 15	p-NPP	0.17 / 74.26	0.23 / 56.88
	bis-p-NPP	0.16 / 26.01	0.33 / 78.02

Table 2*. Effect of light and dark conditions on PMEase and PDEase (μ mol product mg d wt^{-1} h^{-1}) activity of Rivularia strains on days 8 and 16.*

strains	conditions	p-NPP		bis-p-NPP	
		day 8	day 16	day 8	day 16
I	light	0.101±0.005	0.078±0.006	0.045±0.004	0.025±0.005
	dark	0.100±0.004	0.079±0.019	0.045±0.004	0.025±0.004
II	light	0.118±0.009	0.109±0.019	0.107±0.010	0.104±0.010
	dark	0.119±0.02	0.108±0.018	0.108±0.011	0.124±0.012

The difference in V_{max} and K_m values on days 5 and 15 suggests the presence of more than one phosphatase enzymes whether monoesterase or diesterase enzymes in these organisms. It appears that either one enzyme is induced at a later stage than the first one or that both were induced at the same time, but the activity of one was very low at the initial stages of growth and the activity reaches maximum in the later stages of growth when the activity of the first one was nominal. This could account for the different K_m and V_{max} values.

The K_m values of the three strains at day 15 showed less affinity for the substrate. Equal enzymes activity in light and dark suggests that although there are distinct light regimes in rice fields, fluctuations and decreases in light intensity may not affect phosphatase activity in cyanobacteria.

Due to heterogenity of cyanobacteria filaments a rice-field population might be able to form phosphatases in some filaments leading to hydrolysis of organic phosphorus, which in turn would support the growth and development of other filaments. However when the ambient phosphate is entirely organic, cyanobacteria would depend entirely on phosphatases whatever the external concentrations may be.

Unlike some Rivulariaceae strains the two strains studied lack the ability to form hairs. Both strains I and II (non hair-forming) are similar in that the formation of phosphatase activity coincides with the loss of ability to form hormogonia probably these non hair forming strains have an added advantage over the hair forming strain in not wasting cells as a result of hair lyses every time a P-limited trichome encounters a P-rich environment.

Thus phosphatase activity plays a significant role in rice fields by providing phosphorus for the growth of rice plants under unfavorable conditions of P-depletion and under such conditions cyanobacteria may be able to hydrolyze phosphates to support their growth for prolonged periods.

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IMPACT OF REFINERY EFFLUENT ON THE PHYSICOCHEMICAL PROPERTIES OF A WATER BODY IN THE NIGER DELTA

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Abstract. The physicochemical qualities of a refinery effluent and water and sediment of an effluent receiving water body were investigated. The treated refinery effluent contained very high concentrations of phenol (11.06 mg/l), oil and grease (7.52 mg/l), ammonia (8.52 mg/l), COD (91.76 mg/l), TDS (390.6 mg/l) and phosphate (6.2 mg/l), but low in sulphide, nickel, lead, copper and chromium, which were undetectable. High concentrations of phenol (5.13–16.38 mg/l), oil and grease (10.56–15.23 mg/l), and ammonia (4.31–13.17 mg/l) were observed in water and sediment samples respectively, at the point of effluent impact. A high concentration of sulphide (3.74 mg/l) was accumulated in the sediment at the point of impact of the refinery effluent, though it was undetectable in the effluent itself or water sample. The concentrations of these parameters as well as of phosphate, nitrate, zinc and COD declined progressively with distance from the point of impact but were still significantly higher than in control water and sediment in samples 1.5 km downstream from the point of impact. Higher concentrations of the pollutants were recorded in the dry season than rainy season except for phosphate and nitrate, which showed the reverse trend. Nickel, lead, copper, chromium and cyanide were neither detected in the effluent nor impacted water body.
Keywords. *contaminant concentrations, sediment accumulation, seasonal changes*

Introduction

Waste waters released by crude oil-processing and petrochemical industries are characterized by the presence of large quantities of crude oil products, polycyclic and aromatic hydrocarbons, phenols, metal derivatives, surface-active substances, sulfides, naphthylenic acids and other chemicals [22]. Due to the ineffectiveness of purification systems, wastewaters may become seriously dangerous, leading to the accumulation of toxic products in the receiving water bodies with potentially serious consequences on the ecosystem [3, 4].

Various studies have shown positive correlation between pollutions from refinery effluents and the health of aquatic organisms. Previous observations [12] suggested a correlation between contamination of water and sediments with aromatic hydrocarbons from refinery effluents, and compromised fish health. An earlier study [14] demonstrated the accumulation of heavy metals with accompanying histopathology in *Oreochromis niloticus* exposed to treated petroleum refinery effluent from the Nigerian National Petroleum Corporation, Kaduna.

The Niger Delta is host to three of Nigeria's four refineries which generate large quantities of effluents daily. These effluents are discharged into natural water bodies after treatment. Though the compositions of the effluents are regulated by various laws, it is not known whether they comply with the legally accepted toxicant levels for refineries in Nigeria. Furthermore, the impact of these toxicants on the quality of the effluent receiving water body has not been investigated. The present study investigates the quality of the refinery effluent and its impact on the physicochemical quality of Okrika arm of the Bonny River estuary, the body of water that receives the effluent discharged from the NNPC refineries in Port Harcourt.

Materials and methods

Description of the study area

The sampling stations investigated consist of five effluent receiving sites in the Okrika arm of the Bonny River estuary and the control site at Elechi Creek, all in the Niger Delta area. Sampling site I (S1), Ekerikana creek, is the effluent discharge point. Sampling sites II (S2), Okari Creek, and III (S3), Okrika station, are located 500 m and 1000 m respectively from sampling site I. Site IV (S4), Ogoloma, and site V (S5), Edemebiri, are located 500 m each on opposite sides of site III. Sampling site VI (S6), Elechi Creek, close to the University of Science and Technology Port Harcourt, is the reference (control) site, about 35 kilometers from the Okrika station. Human activities like fishing were observed in all the sampling stations except station I. All the stations also receive sewage input except station V (S5). The Okrika stations and Elechi creek of Bonny estuary are of brackish water type, and are mesohaline, with salinity values ranging from 14.4 to 19.1% with a mean value of 17.0%±2.2.

Sample collection

Samples were collected once a month between July 1998 and March 1999. Composite water samples (sub-surface and middle depth) were collected at low tide with a 2 litre plastic hydrobios water sampler and transferred to clean 2 litre polyethylene containers and 250 ml capacity borosilicate glass bottles. Untreated effluent samples include the process waste water (PWW) derieved from the refining process, the raw waste water (RWW) which is a combination of PWW and sewage that is channeled to the rotary bio-disk for treatment. Treated effluents include treated waste water (TWW) which is refinery effluent that has undergone both chemical and biological treatment to eliminate or reduce waste contents to acceptable levels, and the observation pond waste water (OPWW) which is a combination of treated waste water and non-processed waste water. These were collected in polyethylene containers and borosilicate bottles of the same capacity. They were rinsed several times with water or effluent samples at the point of collection. Sediment samples were collected with benthic sampler and transported in polyethylene bags. All samples were transported in ice chests and analyzed for pH and conductivity within 12 hours of collection. Other physicochemical parameters were analyzed later using refrigerated samples.

Water and sediment analysis

An HACH conductivity/TDS meter (Loveland, CO 80539) was used for conductivity and total dissolved solids determination. An HACH pH meter was used for pH determination. An HACH DR/2000 spectrophotometer was used for determination of dissolved oxygen, biochemical oxygen demand, nitrate, ammonia, sulphate and phosphate. An HACH COD reactor was used for chemical oxygen demand determination. All analyses were carried out following the procedures of HACH chemical company (1996). Phenol, oil and grease, alkalinity, chloride/salinity, sulphide and cyanide were measured by methods adapted from *Standard methods for the examination of water and wastewater* [1]. A Perkin Elmer 3100 atomic absorption spectrophotometer (Boston, MA 02118-2512, USA) was used for the determination of the heavy metals including nickel

(Ni), lead (Pb), zinc (Zn), iron (Fe), copper (Cu), and chromium (Cr). Sediment samples were air-dried by thinly spreading on a clean laboratory bench surface at room temperature, and brought to a relatively homogenous state by thoroughly mixing, and sieving with 2 mm mesh before being treated. In the case of fresh samples, large stones and roots were picked out before mixing and weighing. Moisture determination of duplicate samples of the sediment was carried out for dry weight correction. The sediment samples were then prepared (extraction method) for the various physicochemical determination following procedures of Stewart *et al.* [21].

Results

Composition of refinery effluent

The physicochemical qualities of the major effluents generated in the Port Harcourt refineries were investigated, and the level of contaminants estimated. The concentration of the most significant pollutants in the various refinery effluents are shown in *Table 1*.

Process waste water (PWW) is water that is intended to come into contact with hydrocarbons or treating chemicals at a refinery. Raw waste water (RWW) is a combination of PWW and sewage. Treated waste water (TWW) is refinery effluent which has undergone both chemical and biological treatment to eliminate or reduce waste contents to acceptable levels. The observation pond waste water (OPWW) is a combination of treated waste water and non-process waste water. Values represent the mean of seven samples collected over a period of seven months.

PWW contained the highest concentrations of oil and grease (26.42 mg/l), phenol (90 mg/l) and conductivity (1179.4 µS cm^{-1}), and significant quantities of BOD (75.3 mg/l), COD (155.3 mg/l), and ammonia nitrogen (22.35 mg/l). RWW on the other hand contained higher concentrations of BOD (216.0 mg/l), COD (232.1 mg/l) ammonia-nitrogen (26.01 mg/l) as well as the nutrient compounds namely phosphate (15.4 mg/l), nitrate-nitrogen (1.91 mg/l) and sulphate (30.31 mg/l) than the PWW. Treated waste

Table 1. *The physicochemical quality of Port Harcourt refinery effluents (mg/l)*

Parameters	PWW	RWW	TWW	OPWW
pH	07.93	8.3	6.03	8.47
conductivity (µS cm^{-1})	1179.4	1146	774	495.2
BOD	75.3	216	22.08	5.28
COD	155.3	232.1	26.68	91.76
oil and grease	26.42	12.48	4.27	7.52
ammonia (N)	22.35	26.01	13.52	8.52
nitrate (N)	01.53	1.91	1.64	0.754
phosphate	01.11	15.4	6.81	6.21
salinity	19.52	18.62	16.92	13.1
phenol	90	69.112	1.84	11.06
sulphide	<0.01	<0.01	<0.01	<0.01
cyanide	<0.01	<0.01	<0.01	<0.01
sulphate	13.91	30.31	39.08	30.74
TDS	383.6	335.4	209.23	390.6
nickel (Ni)	<0.001	<0.001	<0.001	<0.001
lead (Pb)	<0.01	<0.01	<0.01	<0.01
zinc (Zn)	0.186	0.187	0.11	0.11
iron (Fe)	0.214	0.297	0.203	0.241
copper (Cu)	<0.001	<0.001	<0.001	<0.001
chromium (Cr)	0	0	0	0

water (TWW), showed a reduction in the level of virtually all the parameters observed in RWW, though significant concentrations of phenol (1.84 mg/l), oil and grease (4.27 mg/l), ammonia-nitrogen (13.52 mg/l), BOD (22.08 mg/l) COD (26.68 mg/l) and sulphate (39.08 mg/l) persisted. The OPWW contained high concentrations of the following parameters including COD (91.76 mg/l), oil and grease (7.52 mg/l), phenol (11.06), TDS (390.6 mg/l) and iron (0.241 mg/l) which were observed to be higher than in the TWW. Other parameters remained fairly constant while ammonia nitrogen and BOD showed reduction in concentration. Sulphide, cyanide, nickel, lead, copper and chromium were not detected (<0.001 mg/l) in the refinery effluents before and after treatment.

Contaminant levels at the point of impact of the effluent

The average level of contaminant in the effluent receiving water body within ten meters around the point of effluent discharge, henceforth referred to as the point of impact (POI), is shown in *Table 2*.

Higher concentrations of oil and grease (10.56 mg/l), ammonia nitrogen (4.31 mg/l), phenol (5.13 mg/l) and COD (41.19 mg/l) were observed in the water sample at the POI of the effluent than the OPWW. The concentrations of zinc and sulphate were lower in the water sample than in the OPWW while sulphide and lead were not detected. The concentration of oil and grease (15.23 mg/l), ammonia nitrogen (13.17 mg/l) and phenol (16.38 mg/l) in the sediment samples were much higher than observed in both the river water samples and the OPWW. High levels of sulphide (3.74 mg/l) was observed in the sediments at the POI and represents a significant observation considering that sulphide was neither detected in any of the refinery effluents nor in the river water sample at the point of impact. Zinc was also concentrated many times in the sediment above the values seen in the river water sample and the OPWW.

Variations in the physicochemical properties of water and sediment samples

The physicochemical properties of the effluent receiving water body were evaluated for a distance of approximately 1.5 km from the point of impact of the effluent for 9 months. Mean phenol concentration of 5.13 mg/l was observed at the POI (S1), decreasing rapidly to about 0.006 mg/l at station 5 (about 1.5 km away). The phenol concentration at the control, not impacted water body, station 6 (approximately 35 kilometers away), was approximately 0.001 mg/l (*Fig. 1*).

Table 2. *Comparative chemical quality of the observation pond waste water, water and sediment samples from the point of impact of the Port Harcourt refinery effluents*

parameter (mg/l)	OPWW	samples from the point of impact (POI)		FEPA effluent limitations guideline (1991) mg/l
		water sample	sediment sample	
BOD	5.28	2.76	ND*	10.00
COD	91.76	41.19	ND	40.00
oil and grease	7.52	10.56	15.23	10.00
ammonia (N)	8.52	4.31	13.17	0.20
phenol	11.06	5.13	16.38	0.50
sulphide	<0.01	<0.01	3.74	0.20
lead (Pb)	<0.01	<0.01	0.01	0.05
zinc	0.11	0.05	0.21	NI
phosphate	6.21	0.06	0.14	NI

*ND: not done; NI: not indicated. Values with the 'less than' (<) sign were below detection level.

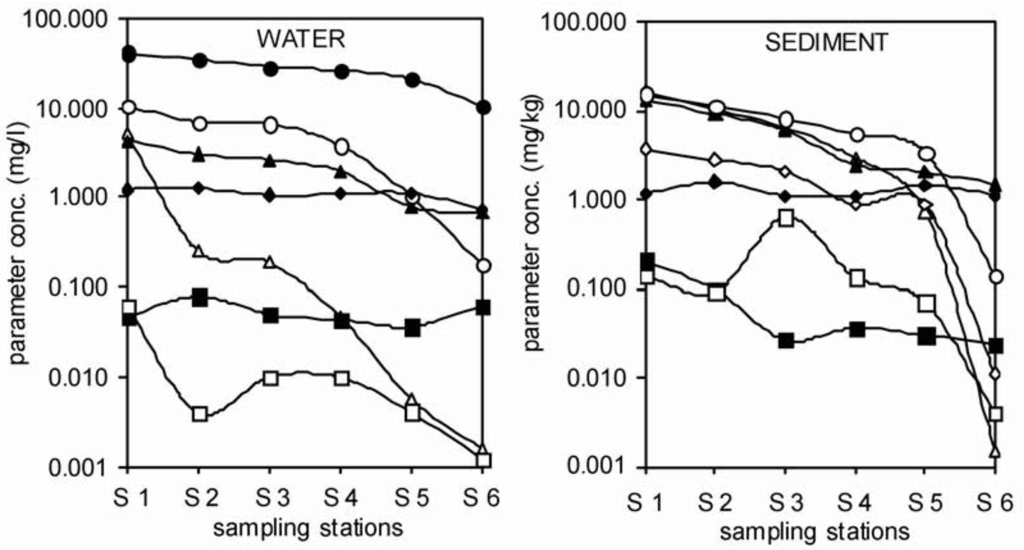

***Figure 1.** Changes in the concentration of NH_4-N (▲), nitrate-N (♦), sulphide (◊), phosphate (□), oil and grease (○), COD (●), zinc (■) and phenol (Δ) in water and sediment obtained along the course (Stations S1-S5) of a refinery effluent polluted water body and from a control site (Station S6)*

Other parameters including ammonia nitrogen, oil and grease, and COD showed similar patterns of variations, with highest values observed at the point of effluent impact and declining rapidly up to a distance of 1.5 km from the POI. The concentrations of these parameters at the control point S6, approximately 35 km upstream of the impacted water body were significantly lower ($p<0.05$) than at the impacted sites. Phosphate and nitrate-N also showed significant decrease in concentration along the course of the impacted water body, but in a less dramatic manner than observed for the other parameters. Zinc showed little or no variation along the course of the impacted water body, with the average values obtained not significantly different from those obtained at the control station S6. Regression analyses of the curves confirm the above observations and showed very strong correlation between concentration and distance from the point of impact for COD, phenol and NH_4-N (R2>0.96) strong correlation for oil and grease (R2 ≈ 0.93), low correlation for nitrate and phosphate (R2 ≈ 0.63) and no correlation for zinc (R2 = 0.05).

The pattern of variation in the concentrations of the sediment quality parameters was generally similar to those observed for the water samples. However, the absolute concentrations of the parameters were higher in the sediment (sometimes up to 10 times) than in the water samples. Significant differences ($P<0.05$) were also observed in the concentration of sediments parameters obtained from the least impacted area and control sites, exceeding tenfold in the cases of phenol, oil and grease and sulphide. The most significant difference in sediment quality parameter was observed for phenol which showed over 300 fold difference between S5 and S6. Another conspicuous case was that of sulphide which, though not detected in any of the refinery effluents or the impacted water sample was found in significant concentrations in the sediment samples and up to 1 mg/l even at a distance of 1.5 km away from the POI. While the concentration of phosphate in the sediment was generally much higher in the impacted water body than the control sampling station, no clear relationship between concentration and distance from the point of impact was observed. Zinc concentration in the sediment showed a more predictable decline from S1 to S5 (R2, 0.75) while nitrate-

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nitrogen on the other hand showed no relationship whatsoever between distance and concentration (R2, 0.0073). Other water and sediment quality parameters investigated including DO, BOD, conductivity, chloride, alkalinity, sulphate, pH, temperature and iron did not show significant difference between the impacted and non impacted sampling stations. Nickel, lead, copper, chromium and cyanide,were not detected in the water and sediment samples of either the impacted or non impacted water bodies.

Seasonal changes in physicochemical parameters

Clearly visible seasonal changes in the concentration of the various water quality parameters were also observed. Generally higher concentrations of the respective parameter were observed in the dry season (December, January, February and March) than in the rainy season (July, August, September, October and November) (*Figs. 2*, *3* and *4*). At the POI (Station 1, *Fig. 2*), phenol showed a seven fold increase in concentration from a relatively low value of 1.007 mg/l in July (the peak rainy season month) to 7.41 mg/l in December (the peak dry season month). A similar trend was observed for stations S2–S6.

However, when the mean value for the dry season months (December to March) were compared to the rainy season months (July to November), statistically significant differences were observed only in stations S1, S2, S3 and S6 ($P<0.05$). A comparable seasonal trend was observed in the concentration of COD, NH_4-N, chloride, zinc, iron, DO, BOD, and oil and grease in the refinery effluent impacted water body (S1–S5) and the non impacted control station (S6). Chloride showed the most consistent variation in concentration with season, with significant differences ($P<0.05$) observed in every one of the six stations sampled. The cases of phosphate and nitrate are worth noting in that they exhibited significantly higher concentrations in the rainy season than the dry season in contrast to the other parameters. Statistically significant seasonal variations in the concentration of the sediment quality parameters were also observed at some of the stations, but these differences were not as evident as in the water samples.

Discussion

Phenol is one of the major pollutants found in refinery effluents. It was found in large concentrations in both the PWW and the RWW. The major sources of phenol in the petroleum refinery PWW are the thermal and catalytic cracking processes. The observed values for phenol of 1.84 mg/l and 11.06 mg/l in the TWW and OPWW respectively were much higher than the 0.5 mg/l [9] or 0.1 mg/l [8] recommended for refinery effluents. High phenol concentration in the TWW was probably associated with the very high concentrations in the RWW that goes in for biological treatment in the rotary bio-disk which probably exceed the design limit. This does not however account for the much higher levels of phenol in the OPWW which was six times higher than in the TWW. This high concentration may be traced to large quantities of non process waste water (NPWW) which is channeled into the observation pond without treatment. Current observations show that the NPWW consist of product tank drains and spent caustic water as well as storm runoffs which contain large quantities of waste matters especially phenol(12.75 mg/l). Phenols have been observed to be very toxic to fish and other aquatic organisms and has a nearly unique property of tainting the taste of fish if present in marine environment in concentration ranges of 0.1 to 1.0 mg/l [5, 6, 20]. The toxic concentration for fishes may range from <0.1 to >100 mg/l, depending on the chemical nature of the phenol, the fish species and the developmental stage, with embryo-larval stages being

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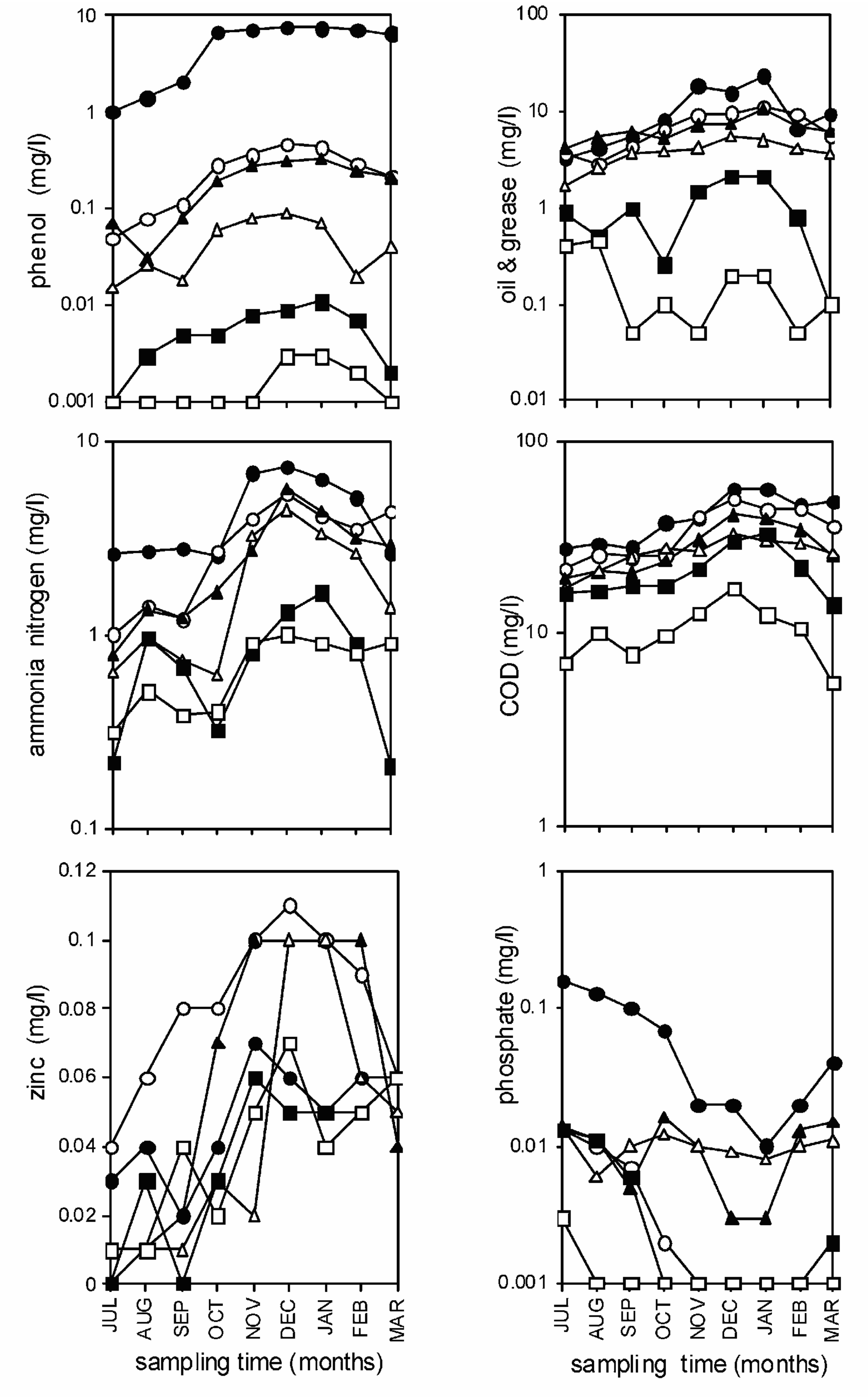

Figure 2. *Seasonal variations in physicochemical parameters of water samples obtained from different sites along a refinery effluent impacted water body* (●: *station 1;* ○: *station 2;* ▲: *station 3;* Δ: *station 4;* ■: *station 5*)*, and a non-impacted control water body* (□: *station 6*)

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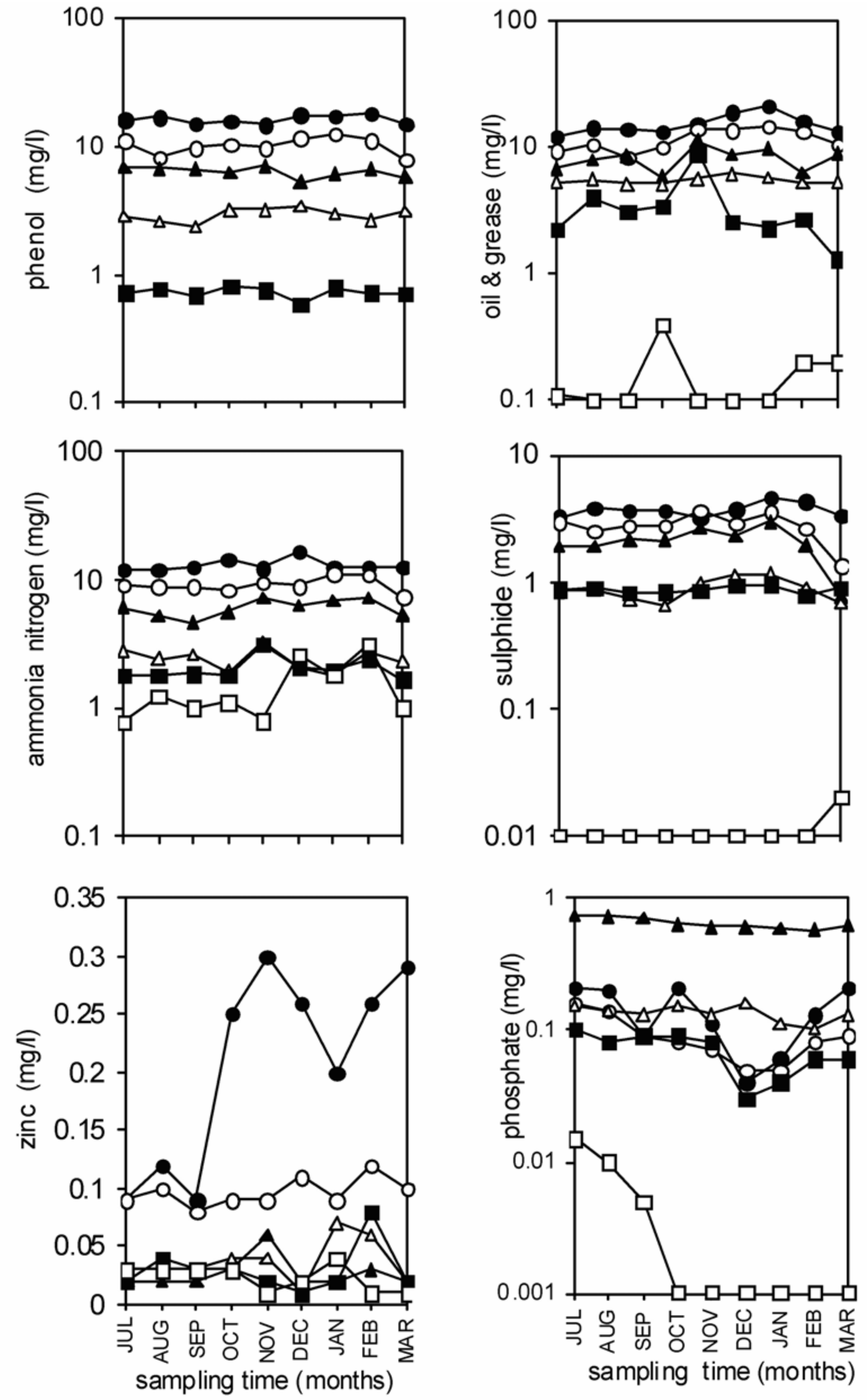

Figure 3. *Seasonal variations in physicochemical parameters of sediment samples obtained from different sites along a refinery effluent impacted water body* (●: *station 1;* ○: *station 2;* ▲: *station 3;* Δ: *station 4;* ■: *station 5*), *and a non impacted control water body* (□: *station 6*)

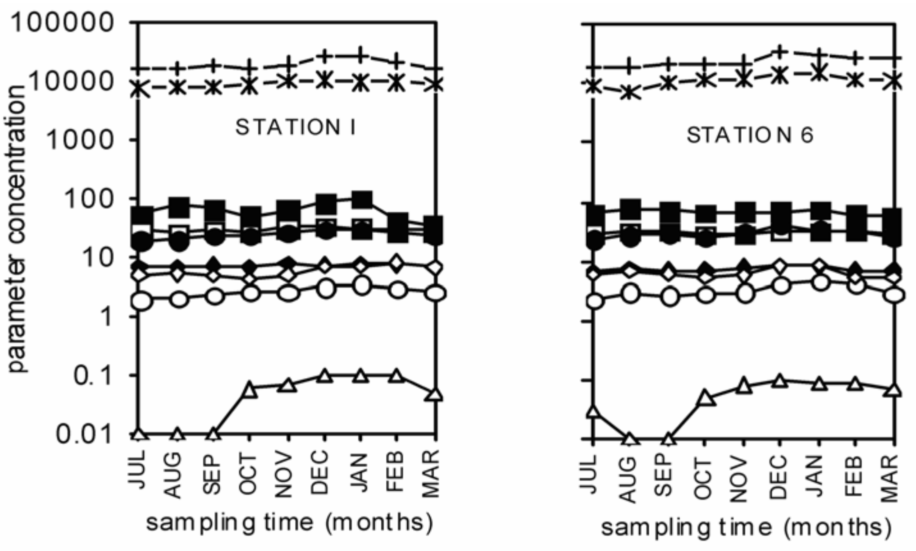

Figure 4. *Seasonal variations in nine physicochemical parameters of water samples obtained from the effluent discharge point (station 1) and non-impacted control water body (station 6) seemingly unaffected by the effluent.* +: *conductivity (µS cm*$^{-1}$*);* ∗: *chloride (mg/l);* ■: *alkalinity (mg/l);* ●: *sulphate (mg/l);* ○: *BOD;* ♦: *pH;* ◊: *DO;* □: *temperature (*^{o}*C);* ∆: *iron (mg/l)*

many times more susceptible than adults [7]. The concentration range of phenol in the sediments (18.02–0.58 mg/l) from the point of impact of the effluent up to a distance of 1.5 km down. Untreated refinery effluents generated by the refinery include the process waste water (PWW) derived from the refining process, and the raw waste water (RWW) which is a combination of PWW and sewage that is channeled to the rotary bio-disk for treatment. The treated effluents include treated waste water (TWW) which is refinery effluent that has undergone both chemical and biological treatment to eliminate or reduce waste contents to acceptable levels, and the observation pond waste water (OPWW) which is a combination of treated waste water and non-processed waste water stream, and is likely to have both acute and chronic effects on the marine organisms, especially the bottom feeders within the impacted areas. Verbal evidence from local fishermen suggests that the area around the point of discharge of the effluents is devoid of fishes and hence no fishing activity is carried out there anymore. Unpublished data also show a dramatic reduction in the number of viable microorganisms found in both water and sediment at the point of impact.

The NPWW was also responsible for the increased concentration of oil and grease in the OPWW and hence for the high level of this contaminant in the effluent receiving water body. The average concentrations of oil and grease in water and sediment (10.56 and 15.23 mg/l, respectively) at the point of discharge were found to be higher than the maximum permissible limit of 10 mg/l for the effluent. In effect, the water quality at the point of effluent discharge may be considered to be similar to an improperly treated effluent that is in need of further treatment action in order to reduce the contaminant concentrations to acceptable levels. Petroleum hydrocarbons have been observed to be toxic to aquatic life. It has been observed [16] that a water-accommodated fraction of crude oil or dispersed crude oil water-accommodated fraction increased the activity of gill citrate synthase, LDH, and hepatic ethoxyresorufin-O-deethylase (EROD) at a concentration of 14.5 mg/l. Lipophilic hydrocarbons have been observed to accumulate

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in the membrane lipid bilayers of microorganisms and interfering with their structural and functional properties [19]. Grant & Briggs [10] concluded that hydrocarbons are the most significant cause of toxicity in sediment samples obtained from around a North Sea oil platform contaminated by large piles of oil based drill cuttings and that polar organic compounds, sulphides and ammonia are of much lower significance. The high oil and grease concentration observed in the effluent receiving water body in this study, in combination with other pollutants, could be responsible for the depletion of the fish and other aquatic life at the point of impact of the effluent.

Considering that natural waters typically contain little ammonia, usually in concentrations below 0.1 mg/l, and that the FEPA and DPR recommended maximum permissible limits in refinery effluents are 0.2 mg/l, the concentration of ammonia in the effluent, water and sediment of 0.21 to 16.8 mg/l were considered to be very high. Concentration of ammonia as low as 0.08 mg/l have been observed to reduce the swimming performance of coho salmon, an effect attributed to metabolic challenges as well as depolarization of white muscle [25]. Another study [23] observed a median lethal concentration (LC50) value of 0.045 mg/l for unionized ammonia (NH_3-N).

Other parameters that were significantly elevated in the water and sediment samples of the impacted water body are zinc, sulphide and phosphate. The concentration of zinc in the sediment is worthy of note as it increased from a basal level of approximately 0.025 mg/l at control sites to about 0.3 mg/l at the point of impact at the peak dry season month of November. Toxicity identification studies have indicated that zinc may be the primary cause of toxicity in certain contaminated aquatic systems [2]. High concentrations of zinc have been observed to be specifically toxic to an aquatic insect *Ranatra elongata* (1.658–2.853 mg/l), and in the microtox test system (1.35 mg/l) [18]. The case of sulphide is very interesting because it was not detected in the effluent nor water samples from the effluent receiving water body, but was found in significant concentrations in the sediment of the impacted water body. This highlights the important role of the sediment as a sink for toxic materials contained in the effluents. As observed earlier [24], contaminants from land-based sources introduced into surface waters rapidly become scavenged by suspended particles that tend to settle to the bottom where they become highly concentrated. The concentrations of phenol, oil and grease, and ammonia were also observed to be higher in the water and sediment at the point of impact than in the effluent from which they were derived. A number of factors may be responsible for this, including evaporation of water resulting in concentration of the toxicants to higher levels than found in the effluent and accumulation over time of trace concentrations of sulphide and other contaminants from the effluent over the 20 year period of operation of the refinery.

The COD value of the effluent discharged into the Okrika River did not meet the effluent limitation standard set by FEPA and DPR for refinery effluents in Nigeria largely as a result of the high COD values of the NPWW which was channeled without treatment into the observation pond. While the graded reduction in the COD values indicated an input from the refinery effluent mainly, the unpredictable distribution of BOD values on the other hand suggest input from different sources, the most important of which could be the faecal waste deposition by the surrounding communities.

Seasonal variations were observed in the concentrations of various water quality parameters, including those whose concentrations were related or unrelated to refinery effluent discharge. While most of the parameters showed higher concentrations during the dry season when water volume is reduced to its minimum two parameters namely, phosphate and nitrate showed reduced concentrations. Phosphates and nitrates are components of agricultural fertilizers commonly used by farmers in the Niger delta area. These fertilizers may be leached into the rivers during the rainy season and thus account for the increased concentration in the Bonny river estuary during the rainy season. Phosphate level in the effluent receiving water body have been observed earlier to be associated with the refinery operation because of the changes in its concentration from the point of refinery effluent. The observed increase in concentration during the rainy season suggests an additional source of this nutrient in the Bonny river estuary, namely the surrounding farmlands. It was noted that urban storm water runoff is a major source of contaminants to southern California's coastal waters and responsible for detectable toxicity in the Ballona creek which drains a highly urbanized watershed [2].

This study has shown that refinery effluent discharged into the Okrika arm of the Bonny River estuary resulted in the presence of high concentrations of pollutant in the water and sediment. The toxicants have been shown to be present in concentrations which may be toxic individually to different aquatic organisms. Pollution of the aquatic ecosystem poses a serious threat to aquatic organisms and ultimately the entire ecosystem. The long term impact of refinery effluent discharge into the Bonny River estuary is not known. An eleven year study of the 1989 Exxon Valdez oil spill revealed that though the contaminants were rapidly eliminated by weathering [15], toxicity of sediments remained at a few heavily polluted locations around the Prince William Sound Alaska, which persisted ten years after the spillage [11]. A recent study [17] has shown that hydrocarbon may remain buried in sediment for up to 30 years without major alteration in concentration. Furthermore, the complex combination of toxic substances that are simultaneously present in refinery effluents may act synergistically and therefore impose a higher toxicity burden on the ecosystem than may be predicted in laboratory studies on individual toxicants [13]. Continued discharge of improperly treated effluent may further compound the worsening environmental problem of the Bonny River estuary, and the delicate ecological balance of the Niger Delta basin. An early resolution of the problem of treatment of the NNPC refinery effluent is therefore imperative in order to save the effluent receiving water body from further degradation.

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STUDY OF MIGRATORY STURGEON CAPTURES IN ROMANIAN SIDE OF DANUBE RIVER
MIGRATION OF FISHES IN ROMANIAN DANUBE RIVER, № 3

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Abstract. Study of fish captures could bring out valuable information on tendencies in stocks dynamics as well as on the level of "fishing pressure" on natural fish population. Decreasing amount of annual captures of marine migratory sturgeons that migrate in Danube River for spawning indicates a decline of natural stocks of these species. Present paper tries to show the main aspects of sturgeons fished in Romanian sector of Danube River, to discuss some relevant aspects that could help people to understand the real issues on this topic better and eventually find the best way to stop the decreasing tendency of wild population of three very interesting species of sturgeons: beluga (*Huso huso* Linnaeus, 1758), Russian sturgeon (*Acipenser gueldenstaedti* Brand, 1833) and stellate sturgeon (*Acipenser stellatus*, Brandt, 1833).
Keywords. *marine sturgeons, migration, capture dynamics, stocks, protection*

Introduction

The worldwide number of sturgeons is estimated about 10 000 to 100 000 adult individuals [2] which probably decreases rapidly. Approximately three quarters of worlds' sturgeon catching depend on only three species: beluga (*Huso huso* Linnaeus, 1758), Russian sturgeon (*Acipenser güldenstaedti* Brand, 1833) and stellate sturgeon (*Acipenser stellatus*, Brandt, 1833). Space distribution of these species is restricted to hydrographical basins of Caspian Sea, Azovean Sea and Black Sea and the main amount of these sturgeons captures belongs to few countries: some states of former Soviet Union, Iran, Turkey, Ukraine, Serbia and Montenegro and Romania. Responding to the decreasing tendency of sturgeon stocks (which is a consequence of over-fishing, losing of reproduction spots because dam constructions and probably changes in fresh water quality – pollution, changes in water level – and habitat degradation in optimal spawning places in rivers), some sturgeon populations from the Caspian Sea and Azovean Sea were artificially preserved through repopulation programs. It is estimated that at the moment more than 30 percent of the total amount of sturgeon spawners in Caspian Sea and almost all adult sturgeons in Azovean Sea are descendants of the individuals introduced by human action [5]. Having a natural access to a large sector of Danube River on almost 1075 km, Romania should also have an active and positive role in working out adequate politics concerning sustainable exploit and preservation of sturgeon's resources in collaboration with all the interested river-side countries. The last couple of decades should be considered as the most unfortunate period of sturgeon-related activities.

Materials and methods

From 2000 to 2001 several expeditionary fishings had been performed, in order to collect samples from different locations on Romanian sector of Lower Danube River. Most of them took place in the middle of migration seasons in the fall and spring and

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also in extra-season, mainly focused on the best places known as preferred sturgeon spawning sites as km 102–103, km 155, km 186, km 23, km 309 and two branches of Danube. We also used data and information from other upstream places. We used special sturgeon drift bottom gillnets and appropriate boats operated by two or three fishermen as catching gears – the same tools and fishing methods used by commercial fishermen. The evaluation of the captures was based on data obtained from both the official national statistics and experimental fishing. We also used a rough method for estimating the number of sturgeons by dividing the amount of mass capture at the estimated individual medium weight for each species of sturgeon. Average individual weight has been calculated in case of 170 individuals: 45 beluga, 50 Russian sturgeon and 75 stellate sturgeon individuals using a representative number for each class of age. We also used some information gathered from individual fishermen, fishing companies and interested non-governmental organizations in order to have a better idea on the size of poaching and black market of sturgeons.

Results

The graph of the total annual amount of sturgeons caught on the Romanian side of Danube River in the last 50 years (*Fig. 1*) shows that the tendency of decreasing captures started in the late 60's.

In the next period, from 1981 to 1993 the quantity of caught sturgeons has been relatively constant with registered values around 50 tons with extremes of maximum 54 tons in 1985 and minimum of 38 tons in 1988.

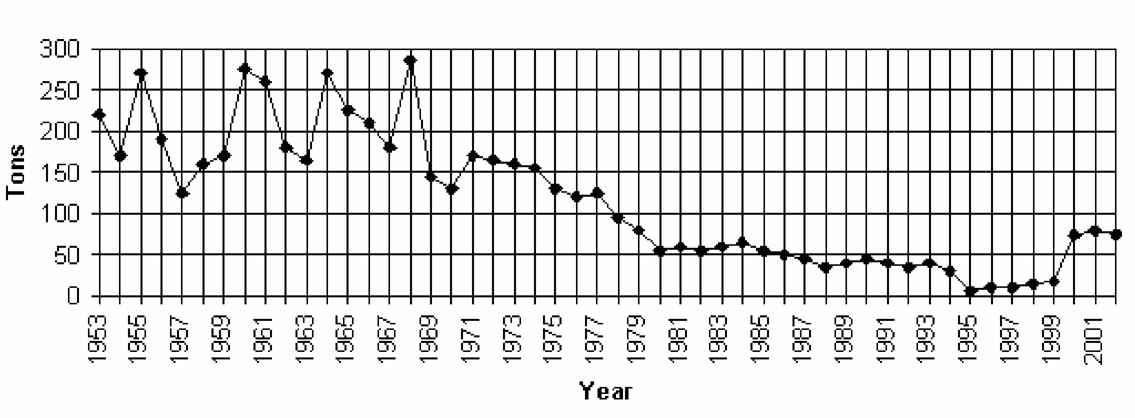

Figure 1. *Sturgeons capture dynamics in Romanian sector of Danube River (1953–2002)*

The official data from the period of 1994–1999 should not be considered as credible amounts. Captures of only 5 to 18 tons registered in that time can not be real. Catches around 60 to 80 tons, that have been registered in the last couple of years of fishing monitoring, could be considered as realistic quantities. However, at this time, the level of yearly captures has dropped almost to the third compared to the 50's and 60's (when the average level of capture exceeded 200 tons per year) and halved if we consider the 70's amounts (average around 150 tons). In the last decade, the trend of decreasing intensity of sturgeons' migration in Danube River should be a consequence of increasing fishing efforts.

Study on the captures of sturgeons in different seasons shows three distinctive peaks registered in February, May and September–October (*Fig. 2*). The maximal values

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corresponding each species of sturgeons are quite different This observation could bring out some information about the yearly intensity of migration, supposing that the fishing effort is constant. There are two aspects to be underlined. First, this observation confirms the existence of two migration periods: one in the spring season that could be significant even earlier, in some winter months and the second in the fall, that starts actually at the end of the summer and has its maximal intensity in September and October. Second, this particular statistical data shows that for all species the peak of capture was registered in the fall season, which brings out the idea that even if sometimes the intensity of fall migration could be higher than that of spring, usually the migration in first part of the year is more important.

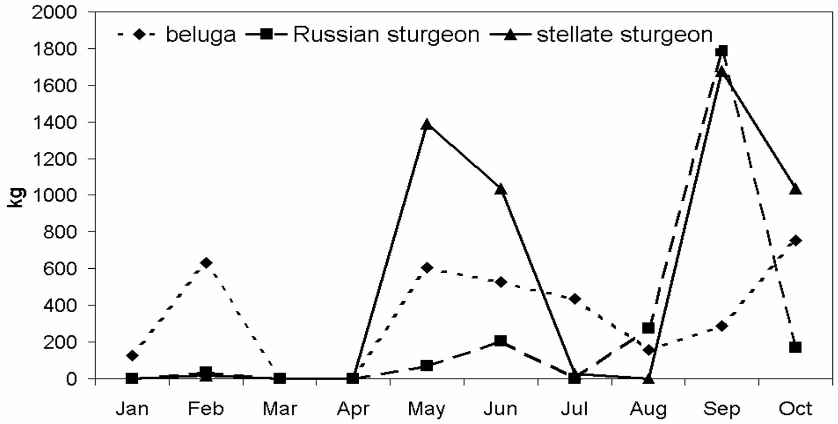

Figure 2. *Monthly dynamics of sturgeons capture in Romanian sector of Danube River*

The different species' relative mass percentage from the total amount of capture registered in 2001 shows a dominancy of beluga (BE): 38.5%, followed by stellate sturgeon (SS): 35.2% and Russian sturgeon (RS): 26.3%. In the last decade, the proportion of these species varied greatly (*Table 1*).

Official statistics did not contain any data regarding to the number of different species of sturgeons caught in the Romanian sector of Danube, so we tried to find out the number of caught individuals belonging to the different species by dividing the mass data with the approximate average values of individual weight (*Table 2*).

Comparing the estimated number of sturgeons in different years, we will find a very different structure of the total annual capture. In 2000/2001, total number of caught sturgeons has been estimated at 7119/7238 (100%) individuals with the following percentage of the different species: beluga 3.1%/3.5%, Russian sturgeon 27.8%/32.1% and stellate sturgeon 69.1%/64.4%. Even if in other years has been small differences, there lies an evident fact that beluga, the most valuable sturgeon, has a very low

Table 1. *Percentage of different species from total amount of yearly sturgeons capture (1992–2001)*

species	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
beluga	24.3	22.4	55.8	43.0	56.6	17.2	57.4	30.7	35.6	38.5
Russian sturgeon	13.7	11.0	32.2	17.2	17.3	39.6	12.8	23.2	24.3	26.3
stellate sturgeon	62.0	66.6	18.0	38.8	26.1	43.2	29.8	46.1	40.1	35.2

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Table 2. *Number of sturgeons estimated on conventional average weight and the mass amount of capture (2000–2001)*

species	capture (kg) 2000	capture (kg) 2001	average weight (kg)	average fish number 2000	average fish number 2001
beluga	26182	30623	120	218	255
Russian sturgeon	17842.5	20936	9	1982	2326
stellate sturgeon	29513.5	27940	6	4919	4657

proportion: about 3–4% in the structure of all sturgeons caught in Low Danube River. It is supposed that their stocks in Black Sea has been also decreased. During the 2000 and 2001 expeditionary fishing campaign we have caught a total number of 170 sturgeons. Structure of this experimental capture has been relatively close to the estimated one: beluga: 2.9%, Russian sturgeon: 25.1 and stellate sturgeon: 72.0%. This confirms the accuracy of our estimation based on official statistic data. Fish meat of all species of sturgeons is quite appreciated on the Romanian market, but their roes are the most valuable: the commercial product is usually called “caviar”, which is a luxurious product worldwide. Because the credibility of statistical data regarding the real amount of caviar obtained from sturgeon captures is very low, we also tried to estimate these quantities (*Tab.3*) using biological information on sex ratio and average weight of female gonads.

Table 3. *Estimation of the amount of caviar in total yearly capture of sturgeons in 2000 and 2001.*

species	estimated fish number 2000	estimated fish number 2001	sex ratio	estimated females number 2000	estimated females number 2001	average gonad weight (kg)	roes (caviar) (kg) 2000	roes (caviar) (kg) 2001
beluga	218	255	0.44	96	112	20.0	1920	2240
Russian sturgeon	1982	2326	0.54	1070	1256	2.3	2461	2888.8
stellate sturgeon	4919	4657	0.50	2460	2329	1.4	3444	3260.6
total	7119	7238	—	3626	3697	—	7825	8389.4

As a result, our estimate is that the total amount of caviar obtained from sturgeons caught in Romanian side of Danube should be about 8 tons per year and beluga caviar (the most appreciate caviar) represents approximately 25% of total. These values do not evidently include the amount of poaching roughly estimate at about 30–40% of total annual capture.

Discussion

Beluga is considered to be the most valuable migratory sturgeon that comes in Danube River only for reproduction. Older individuals prefer an earlier migration in spring season that starts in the winter months. Medium sized individuals migrate mainly in the fall, and the younger spawners migrate at the end of spring. Russian sturgeon migrates upstream Danube River in the fall and spring but the most important season are the spring months (March–May) rather than fall (September–October) [6]. However, we have found that at least some times the migration in the fall could be more intense. In the spring time, the migration may last till late June and because this species is well adapted to fresh water, a group of biologists consider, that some biological forms of Russian sturgeon remain all year long in Danube River [4]. Stellate sturgeon swims upstream for reproducing either in the fall or in the early spring. It is supposed that individuals that migrate in the fall will spawn also in the spring [3] because their degree of gonad maturation is inferior compared with the adults that migrate in the spring (usually at the beginning of March). The spawning usually takes place at the end of May in deep locations with a high water current. There have been sites which juvenile individuals use to crowd for feeding such as some spots from km 16 to km 60 on the Branch of Sfântu Gheorghe and also – but not so often, in some sites from km 770 to km 780, situated upstream. Analyzing all data and information available on all three species of sturgeons it can be found that different sized juveniles (till 14.5 cm and 45 gram) remain in Danube River for longer periods which demonstrates a long spawning time and also that maybe not all fish arrived in the spring return in the same year for feeding into Black Sea. Some of the sturgeon juveniles could stay for passing winter season in the favourable areas of Danube or Danube River Branches. The study of the captures brings out interesting information concerning the intensity of the migration if we consider a relatively constant fishing effort. Between 1950 and 1975, Romanian official statistics showed an amount of capture that ranged from 150 tons to 290 tons per year, the average of that period being almost 230 tons compared to the total quantity to be less than 80 tons per year in 2000 and 2001. The annual amount of capture began to decrease in the middle of 70's. This is the period, when the building of hydro energetic plants "Iron Gates" on Danube River started (1969) and the plant started to work (in 1975). Therefore the free access to the best reproduction sites became inaccessible to the sturgeons, After all, these huge dams did not have specially designed passing ways or similar facilities that would allow sturgeons to pass upstream. As a result, captures have probably started to drop to the unusual low levels in the decade started in 1969. The decreasing tendency of captures remains an evident fact until 1980. In this period the amount of the annual capture have dropped from about 170 tons in 1971 to 52 tons in 1980. From 1981 to 1992 registered data show a relatively constant period from the point of view of annual capture, with a low average capture level of about 50 tons, but no more than 60 tons. After that period statistic data apparently indicate a tremendous fall of the sturgeons capture. From 1994 to 1999 there are registered values of only 5 to maximum 18 tons. That could be considered as a catastrophic decline of sturgeons' migration, but in our opinion these data should be treated with caution. Official statistics lack the information about the real amount of capture and are of a very low credibility. In the last years there has been an evident tendency of increasing capture: in 2000 and 2001: the level of annual captures were about 72–73 tons. Not having any certain data regarding changes in the fishing effort there is a risky idea supposing a natural increase of sturgeon stocks. Our unverified information drives to the supposition of a higher fishing effort than before, as a result of less respecting rules and regulations on fishing and also inadequate control and security on this field. It is interesting to underline that recent age and size structures of all species are quite different when compared actual data with older information. Ecological meaning of this fact is not very clear: it could be either a start of the enhancement of sturgeon populations that migrate in Danube River for reproduction or par contraire one more indication of the real tendency of decreasing number of mature individuals.

Conclusions

The main reason of stocks decreasing in the last years relates to the long social, economic and political crisis that involves an increasing number of fishermen into legal or

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illegal sturgeons catching, which means a lesser respect of fishing rules and a very active black market for fish products. To these factors, the less quantified effects of Iron Gates dams, hydrologic regulation buildings, pollution and the bad management of sturgeons' fishery have to be added. As a result, the annual capture of sturgeons dropped dramatically. There is clear evidence of rapidly decreasing amount of yearly capture of sturgeons in Romanian Danube River in the last few decades. As the statistic data show, the annual level of sturgeon's capture has been dramatically decreasing since 1975, the year in which hydro energetic plant "Iron Gates II" on Danube River started to work. Also, the dam had been significantly modified the hydrological dynamics of Danube River as well upstream and downstream with a major change of local biotopes and aquatic communities. Even when the credibility of last decade's statistic data are very low and also other information on the annual level of sturgeon's capture are quite questionable, there is a certain tendency that the number of the mature individuals of these species that come into Danube River for reproduction are decreasing and the individuals still coming become smaller and younger. Because we do not have any related data on sturgeon populations in Black Sea, it could only be supposed that the major causes of this tendency are important habitat changes related to the way of migration and spawning sites, high pressure of commercial fishing, the very high level of poaching and also (maybe) environmental modifications in Black Sea ecosystem related to nutritional spectrum of sturgeons over there.

For the better knowing of the dynamics of main species of sturgeons that enter Danube River for reproduction, there is a need of more focused studies to be done on this topic, not only from Romanian part but also by other riverside countries, to cooperate with all available data on their ecology in the frame of a wide open strategy concerning monitoring, mitigation and stock protection policies, and mainly, to efficiently put in practice the adequate strategy.

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SEASONAL DYNAMIC PATTERN ANALYSIS IN SERVICE OF CLIMATE CHANGE RESEARCH
A METHODICAL CASE-STUDY — MONITORING AND SIMULATION BASED ON AN AQUATIC INSECT COMMUNITY

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Abstract. Our aim was to approach an important and well-investigable phenomenon – connected to a relatively simple but real field situation – in such a way, that the results of field observations could be directly comparable with the predictions of a simulation model-system which uses a simple mathematical apparatus and to simultaneously gain such a hypothesis-system, which creates the theoretical opportunity for a later experimental series of studies. As a phenomenon of the study, we chose the seasonal coenological changes of aquatic and semiaquatic Heteroptera community. Based on the observed data, we developed such an ecological model-system, which is suitable for generating realistic patterns highly resembling to the observed temporal patterns, and by the help of which predictions can be given to alternative situations of climatic circumstances not experienced before (e.g. climate changes), and furthermore; which can simulate experimental circumstances. The stable coenological state-plane, which was constructed based on the principle of indirect ordination is suitable for unified handling of data series of monitoring and simulation, and also fits for their comparison. On the state-plane, such deviations of empirical and model-generated data can be observed and analysed, which could otherwise remain hidden.
Keywords: *state-planes, NMDS, ordination, seasonality, Heteroptera, similarity patterns*

Introduction and objectives

Discovering the structure and operational mechanisms of aquatic communities and exploring the basic pattern generating processes is a scientific task standing in the frontline of ecological research; it has an extraordinary significance both from the aspect of basic and applied research. Thorough knowledge of the temporary state and the changes occurring in our environment is inevitable to prudently coordinate our society-level activities. Examining the state of living communities which the Biosphere contains, tracing the changes in their state (monitoring), evaluating data structures and researching the affecting factors standing behind ecological patterns can be mentioned among the most important objectives from the aspect of the long-term interests of human society [105].

Concerning the methodical and methodological trends of ecological research, three main approaches can be distinguished.

Field ecologists who start from the observation of real natural processes are striving during their examinations to cause the less disturbation to these processes [94, 115, 159, 176]. Their task is the prejudication-free description of synbiological patterns and in the possession of these precise descriptions (data series and tables) they try to explore the affecting factors (or to be more precise: their background patterns) generating the observed patterns. To achieve this, they generally apply the multivariate data-structure explorative methods and other, often used canonical methods of pattern exploration [171, 152]. The most significant theoretical description of this methodology is attributed – even by international comparison – to the works of Juhász-Nagy [88, 89, 90] and his followers.

Another school of ecological research does not concentrate on the complex description of natural processes, but stands a hypothesis connected to a selected part-phenomenon or a hypothesis-system consisting of some alternative hypotheses to the focus. The point of these works is to test the differentiating predictions of hypotheses – often during firmly controlled, manipulative experiments. In the evaluation of these experiments, the traditional possibilities of trial statistics and variance analysis are exploited [45, 139]. Often cited classics of „anglo-saxon ecology" followed this way in many cases [147].

The third main trend is represented by modelling (theoretical) ecologists who – in the possession of well-described basic biological phenomena and with the application of the most possible hypotheses – construct a very precise mathematical description (model) of the simplest theory concerning the observed phenomenon. The point of this methodology can be described as a logic cycle, which consists of testing (confronting the model with case studies) and developing (upgrading and re-constructing) the model. By applying this methodology, more and more realistic theory of the observed phenomenon can be gained, but in the initial stage of the work only a fragment of the available knowledge is used. The leading studies and handbooks of exact theoretical ecology use ecological model-systems as main guidelines, the results of the other two approaches mentioned above are used in many cases only as illustrations. However, the constructed models available these days are quite far from the observed results of field ecologists [87].

All three approaches detailed above have some obvious advantages and disadvantages. Reliable, prudently checked and justified knowledge can be obtained most easily by evaluating experimental situations. But very often the criteria of prudent checking either excessively narrows the valid circle of the statement or limits the complexity of the phenomena which can be examined this way. By the help of this methodology, such results can be obtained relatively easily which are justified professionally at quite high quality, however these are of low heuristic power and far from practical availability. Thus, correct observation and detailed description is inevitable for studying complex – and from pratical aspect potentially important – phenomena, since it is impossible to draw realistic hypotheses without reliable basic data. Problem is that field ecology studies which try to examine communities existing among or close to natural circumstances in complex approach, are forced to stay at gaining basic data or – at most – exploring simple correlations, because during reasoning such complicated hypotheses should be drawn, by which testing would prove to be an absolutely hopeless effort. By reasonal examination of complex phenomena, the description of hypotheses in simulation models can not be avoided, since without them we wouldn't be able to predictively differentiate between alternative explanations. Another advantage of simulation technique is that it reflects those interpretational errors, which can be disregarded even at the stage of unifying those part-theories which are separately justified or at the stage of statistical evaluation.

Ecology, as a separate field of science was named by Haeckel in 1866, but at that time he meant to a some extent different discipline under this name: a physiological field of studies which examines the connection of living organisms and their environment – source: [109]. However, it has to be admitted, that a bit later he also formed a more sophisticated opinion:

„By ecology we mean the body of knowledge concerning the economy of nature – the investigation of the total relations of the animal both to its inorganic and to its organic

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environment; including, above all, its friendly and inimical relations with those animals and plants with which it comes directly or indirectly into contact – in a word, ecology is the study of all those complex interrelations referred to by Darwin as the conditions of the struggle for existence" (Ernst Haeckel 1870, source: [1]).

Basics of supraindividual biology – which in todays professional term is now regarded as „ecology" – were laid down in the works of Clements [34, 35], Voltera [199, 200], Lotka [104], Elton [42], Gause [52], Lindeman [101, 102], and Allee [1, 2, 3], in the first half of the 20th century. In the early works, results of the three methodical ways detailed above were present supporting each other, and in unity. However, the three roots of the different methodical schools existed even at the dawn of ecology, so none of them can be considered as older or newer than the others.

According to the corporal point of view of the Ecological Committee of the Hungarian Academy of Science, ecology:

„has the task to research those limiting (...) phenomena and prosesses (...), which directly control the behaviour and spatial-temporal quantitative distribution of populations and their communities." [5].

So the corporal point of view leaves the methodical problem open. Up to now, this methodical specialization has grown to such an extent, that the representatives of the different ecological schools often can not even comprehend each others works and they group in separate organisations, publish in different papers and use substantially different terms.

Specialist field researchers often have the opinion that taxonomical and faunistical exploration of nearly natural communities (but even agro-ecosystems and other monocultures as well) is at such a low level, that forming of operational hypotheses or models makes completely no sense. According to them, many decades have to pass until data collecting and description can be exceeded. Followers of the experimental methodology think however, that serious scientific research can be conducted only in the case if there is a clear "professional hypothesis" before the start of the work. If this is the only way, we should examine even dramatically simplified experimental situations, but we have to strive to gain scientifically proven knowledge. Many modelling ecologists ("strategic modellists" or "theoretical ecologists") tries to get a grasp on basic phenomena and to study the theoretical possibilities. According to them, the development of the methodology of ecological modelling is the most important step. "Tactical modellists" or "applied ecologists" concentrate exclusively on the prognostical usefulness of the model, and they do not even care about comprehensibility (biological interpretability of mathematical expressions) of the models.

Although the above-mentioned theories are seemingly fundamentally contradictory to each other, each of them is logical in itself, and in its right place acceptable.

During our research which led to this current work, our aim was to try to approach an important and well-studiable phenomenon – connected to a relatively simple but real field situation – in such a way, that the results of field observations could be directly compared with the predictions of a simulational model-system which uses a simple mathematical apparatus and, simultaneously gaining such a hypothesis-system, which creates the theoretical opportunity for a later experimental series of studies.

Our opinion is that it is worth dealing with the approach of the three methodical schools during real case studies – even if the initial research results are obviously less detailed from the point of describing, less developed from the aspect of modelling and less controlled compared to experimental methodology – since working out of a unified methodical framework can be expected only from series of real case studies.

For the purpose of the above-mentioned methodical case study, we chose – based on detailed comparative pre-examinations [62, 64, 65, 66, 74] – a section with the most stable water level of the Szilas stream at Budapest. This part of the stream can be found right under Lake Naplás, its basin is dug and grassed, and the surrounding transect of the basin has nearly natural flora. As a phenomenon of the study, we chose the seasonal coenological changes of aquatic and semiaquatic community of Heteroptera species. During pre-examinations the Szilas stream and its side-waters were from their source to destination thoroughly surveyed and preliminary examinations have been made. In the text of the current work, the expression "Szilas stream" means exclusively the section marked for detailed studies and its state between 1991 and 2002.

For a phenomenon to study, we chose the seasonal coenological changes in the state of the aquatic and semiaquatic Heteroptera community.

Direct objectives of our work in accordance with the above-mentioned facts can be summarized as follows.

1. Working out of such a methodical case study, which synthetises, tests and develops the field- and simulational methods suitable for investigating the seasonal changes of aquatic communities.
2. Description of the seasonal changes of the Heteroptera community and exploration of the main principles of the process on the observed section of Szilas stream.
3. Developing such an ecological model-system, which is suitable for generating realistic patterns highly resembling to the observed temporal patterns, and by the help of which predictions can be given to alternative situations of climatic circumstances not experienced before (e. g. climatic changes), and furthermore which can simulate experimental circumstances.

Review of literature

The research of aquatic and semiaquatic Heteroptera has a significant history in Hungary from the beginning of the XX. century to our days but the publications are mainly taxonomic and faunistical issues. We know very little about these species from ecological and zoocoenological aspects, however recently complex ecosystem observations took place in some of their biotops [189]. These before-mentioned facts even in themselves would underline the importance of such examinations. In addition, some publications mention that in certain aquatic habitats these animals might have outstanding ecological significance simply due to their quantitative proportions [120]. But maybe even more important is the fact that the majority of them are predators, so they are near to the end of the foodchain of aquatic communities [9]. Such living organisms – based on numerous experiences – could be relatively sensitive for even tiny or (for us) hidden changes in their environment. These attributes render them especially suitable for (following the principle of bio-indication) tracing the changes in their environment and to gather important information on their habitats [72, 73]. Besides, certain aquatic Heteroptera species bear huge economical significance being fish pests and/or fish feed. Moreover, the possible role of some semiaquatic species in public health [117] and plant protection [158] can be also interesting.

One of the most important pattern-generating factor of the moderate climate nearly natural ecosystems is seasonality and the seasonal dynamism of the community structure which follows it. Based on database findings of the National Light-Trap Network for Plant Protection and Forestry seasonal dynamism hides the pattern generating role of

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the spatial differences in habitats and also of the so-called interannual effects [70, 71, 129, 126], which means that seasonal differences on the same collection spot in the same year are much greater than that can be generally perceived on the same aspects of different years on different Hungarian habitats. Results of Schmera [167, 168] also prove this, who examined the flying activity patterns of a selected Trichoptera species and couldn't find great differences between the sampling spots. Patterns were synchronized but significant differences could be observed between months. Work of Ayre & Lamb [7] support the theory of lesser significance of annuality.

In aquatic ecosystems, the connection of seasonality and eutrophisation has been examined mainly from system-ecological aspect [57].

Seasonal dynamic examinations of Heteroptera communities in aquatic habitats

Regrettably, both domestic and international literature lack works which analysed the seasonal dynamic patterns of coenologic state changes of aquatic Heteroptera communities. However, basic data bearing information on seasonal appearance patterns of aquatic Heteroptera or dealing with seasonal part-phenomena or seasonal characteristics of certain species are available. The most detailed general ecological summaries can be found in the works of Savage [162, 163, 164, 166] and Møller-Andersen [121] for the Western-European species. By studying these works, a picture from the main disciplines generally characteristic to Gerro- and Nepomorphans can be gained. There can be found basic data suitable for coenological and seasonal dynamic analysis in the work of Green [53] from the macroinvertebrata (even Heteroptera) fauna of three Great-Britain ponds, however, the paper does not deal with the interpretation of patterns.

There are many publications dealing with different bioindicational issues, thus indirectly pointing on factors and phenomena influencing seasonal patterns. There are also known for a long time such kind of efforts, which try to describe individual habitats by aquatic and semiaquatic Heteroptera communities. Among others, Fairbairn [46], Spence [177, 178, 179], Nummelin & *al.* [127], Vepsäläinen & Nummelin [198], Oscarson [131] and Vásárhelyi [191] also deals with habitat selection. Connecions between habitat, population structure and seasonality are examined in the [202] work of Zimmermann through the life-cycle of the semiaquatic Heteroptera named *Mesovelia furcata* which also lives in Hungary. Similar examinations has been conducted by Brönmark & *al.* [29] on the *Velia* species in Swedish streams. Macan [106] indicated correlation among species composition of the aquatic Heteroptera, certain chemical parameters of lakes and the quantity of coastal vegetation. Savage & Pratt [165] in an early study couldn't find any correlation among certain chemical parameters and aquatic Heteroptera communities. Later, however, significant influence of water conductivity has been pointed out [161, 162]. The pH of the water has also a determining effect on the species composition of the aquatic Heteroptera communities [44]. Effects of pH and temperature are evaluated in the 1996 paper of Blacchi & *al.* based on faunistical data series on aquatic and semiaquatic Heteroptera living in Italy. Macan [107] found tight correlation between the quantity of organic matter accumulating in lakes and the aquatic Heteroptera species and communities. Bröring & Niedringhaus [30, 31] pointed out correlation between the type of the examined lakes and the Heteroptera species which can be found in them. In recent times multivariate statistical methods were also applied in the exploration of the structure of aquatic and semiaquatic Heteroptera communities [48, 49, 50, 70, 72, 73]. These groups were found not to be quite suitable for water characteization by Eyre & Foster [44].

Jansson [84] in his study indicated the role of three species belonging to subfamily Micronectinae in the water quality of Finnish lakes. Sládecek & Sládeckova [172] worked out the saprobiological indexes of Central European Corixidae species. These are held valuable by Savage [164] for the application for water qualification purposes. Correlation of seasonal phenomena and hidrobiological features are dealt with in the works of Pandit & *al.* [135, 136, 137] concerning Indian currents and also at DuBois & Rackouski [41] in connection with North American still-water habitats.

Numerous papers deal with the life-cycles and phenological phases of certain, individual species based on both field and laboratory examinations, however, most of them primarily concentrate on species which aren't distributed in Central Europe [33, 39, 85, 86, 111, 125, 123, 130, 160]. In the work of López & *al.* [103], phenology, larval stages, certain quantitative morphological characteristcs and some water-chemical parameters of the collection sites of a *Sigara* species prevalent in the Iberian Peninsula have been evaluated. The study of Pajunen & Jansson [134] measures seasonal changes of sex ratio concerning still-water Corixidae, and this paper also serves as a useful methodological work for modelling. Packauskas & McPherson [133], McPherson [114], McPherson & *al.* [112, 113] and Kaitala [92] conducted thorough experimental, controlled in vitro life-cycle studies.

There have been also published important studies in recent years in connection with ecophysiological mechanisms which are in the background of seasonal-dynamic patterns [22, 27, 95]. Results of this kind could prove useful later by interpreting processes or developing models.

Quite interesting, that numerous papers deal with the seasonal aspects of wing polymorphism [4, 54, 170, 179, 201, 124]. Field data show that this characteristic should be considered by fine-tuning of models. The amazingly thorough study of Vepsäläinen [197] deals with seasonality, wing polymorphism and correlations between pigmentation and habitat, which has been conducted on *Gerris* species during his short visit in Hungary.

There isn't any summarising work for Hungarian species yet. Comments based on collecting experiences can be found in the works of Soós [175] and Benedek [21]. The most detailed and summarized work about Nepomorphs is undoubtedly the work of Bakonyi [9] – this study also includes coenological, phenological and population genetic chapters – however it does not deal with semiaquatic Heteroptera. Faunistical notes from the area of Hungary casually include data applied for different dates of colletction, but these can be considered generally sporadic. Data from the most thoroughly explored areas published by different authors are generally simple species lists [10, 11, 12, 13, 14, 23, 24, 25, 36, 47, 58, 59, 62, 64, 74, 173, 183, 186, 190, 192, 194]. This is because explorative faunistical studies have generally been conducted for completely different purposes, and even if there some other data emerged, there haven't been published directly. Fortunately some counter-examples can be also found [119, 120].

On the present level of our knowledge and available data, the „museum method" worked out by Soós [174] and further developed by Vásárhelyi [187] provides reliable information on phenological characteristics of individual domestic species. With this method, characteristics of aquatic and semiaquatic Heteroptera species have been described by Benedek [19], Benedek & Jászai [20] and Bakonyi [9]. Based on our own field observations, predictions of the „museum method" coincide quite well – in some cases highly above the expected level – with data from detailed quantitative measurements.

We dealt with seasonal-dynamic patterns of Heteroptera communities in publications [71, 75, 78, 129, 132, 155, 156, 193]. Seasonal-dynamic and phenologic patterns of other insects and insect communities are also detailed in some of our other publications and by working up models, the experiences of these works have been also utilized: [75, 78, 129, 132, 150, 193]. We usually only cited these basic data which were used for evaluating seasonal-dynamic patterns and were published generally in faunistical notes and in our papers dealing with pattern evaluation. We reported about Heteroptera in our following data-announcing papers: [37, 38, 62, 63, 64, 65, 66, 67, 74, 183, 184]. Other publication related to Heteroptera: [76]. Basic faunistical data on other insects used for seasonal analyses are included in the following papers: [25, 40, 153, 154, 155, 156, 157].

Materials and methods

The examined section of the Szilas stream as a habitat

A most detailed definition of the characteristic features of a habitat selected for the purpose of an ecological case-study is crucial to create a firm base for comparisons with other observations carried out (maybe later) by others, on other habitats. Regrettably, exploration of domestic small water currents is at very low level (on 90% of the currents not any kind of meritable examinations have taken place until recently). Thus, in this part we could rely only on the results of the pre-examinations and some literature sources.

A decisive proportion of our field studies published in this paper has been carried out between 1991 and 2002 on a (Danube-side) section of Szilas stream, which lies next to the Naplás Lake of Budapest (former Szilas stream reservoir). The Szilas stream is a left-side tributary stream of Danube, which flows through the Pest Alluvial Plain. The area can be found on the northeastern part of Budapest, at the eastern border of the XVI. District. The relatively stable hydrological properties of the section in question are guaranteed by Naplás Lake (or the leaking of its dam). The work has been started with the practical environment protection examination series for preserving natural values of Naplás Lake and its surroundings. As a result of the series of examinations, the most valuable parts of the area became protected. The geomorphological characteristics of the landscape are determined mainly by the 400–500 m wide alluvium of the stream and the V. Danube terrace which is more gently sloping on the Northern and steeper on the Southern side and consist mainly from grave and sand [91, 140, 141, 169]. The Szilas stream appeared in the Pleistocene era. The flow direction of the stream was shaped to gain its present characteristics during the effects of Middle-Würm movements. At the beginning of the Holocene era, the Szilas stream formed two narow terraces, huminite molding slime being the main material of its alluvium [180].

From climatic aspect, the area can be considered as moderately warm and dry, with a slightly less than 2000 sunlight hours (1800 hours in summer and 180 in the winter). Annual average temperature is 10.0–10.2 °C, length of frost-free period varies between 188 and 219 days. Historical average of maximum temperature varies between 34.0–34.2 °C, minimum temperature betwen -14.5–16.5 °C. Daily average temperature between April 10th and October 19th exceeds +10 °C, this means 190–192 days annually. Annual sum of precipitation is approximately 550–600 mm, 310–340 mm in the vegetation period. 30–33 snow-covered days are presumable, average maximum snow thickness reaches 20 cm. Aridity index moves between 1.17 and 1.28. Most prevalent wind direction is NW, average wind speed is between 2.5–3 m/s [110].

On the section in present study, average width of the stream is between 150–250 cm, average depth in the current line is 8–35 cm, with a current speed of 0.01–0.4 m/s in the

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vegetation terminal (half year) of the examination period. Vegetation cover of the length of the bank line 40–100%, and vegetation coverage of the water surface varied between 30–100%, shading of the foliage above the water-course under 1%. By our own measurements, pH of the water is between 7.8–8.3, conductivity 0.79–1.90 mS/cm, chemical oxygen demand (KOI_{Mn}) 3.2–15.0 mg O/dm^3, level of dissolved oxygen 70–100%, algae count 0.3–3.5·10^6 (individual/dm^3). We measured by coloritmetric rapid tests the nitrate concentration between 10–25 mg/dm^3, nitrite 0.05–0.25 mg/dm^3, phosphate 0.1–0.6 mg/dm^3. We couldn't detect ammonia with rapid test. Further chemical and hydrological data were published in [74]. From hydrobiological aspect, the water can be considered mesosaprobic, meso-eutrophic and mesohalobic.

From botanical aspect, the valley of Szilas stream lies on the border of two flora regions, Matricum and Eupannonicum. Vegetation and plant-coenological characteristics are described in the works of Borbás [28] and Rajkai [148, 149]. The vegetation of the immediate surroundings of the examined section has been described in detail by Stollmayer [180], vegetation of the water-course by Hufnagel & Stollmayer [74]. Vegetation of the examined section is mainly characterised by *Sium latifolium* L., *Myosotis palustris* (L) Nath. em. Rchb., *Mentha longifolia* (L) Nath., *Mentha aquatica* L., *Juncus inflexus* L., *Scirpus sylvaticus* L., and *Carex acutiformis* Ehrh.

The fish fauna of the section has been examined by T. Erős. According to his (hitherto unpublished) data, on a large proportion of the area, fish hardly can be found. Based on his thorough examinations, in the deeper parts of the course small numbers of crucian carp (*Carassius carassius*), spined loach (*Cobitis elongatoides* complex), roach (*Rutilus rutilus*), European chub (*Leuciscus cephalus*), black bullhead (*Ameiurus melas*), bleak (*Alburnus alburnus*), European perch (*Perca fluviatilis*), pumpkinseed (*Lepomis gibbosus*) and Chinese rasbora (*Pseudorazbora parva*) can be found.

According to the number of individuals, 89% of the fish belong to the omnivor, 5% to insectivor-piscivor, 4% to insectivor-detritivor, 2% to insectivor feed biology group. Most prevalent species is the crucian carp.

According to literature data [93, 180] in the vicinity of Lake Naplás, numerous amphibious species, like smooth newt (*Triturus vulgaris*), crested newt (*T. cristatus*), fire-bellied toad (*Bombina bombina*), common tree frog (*Hyla arborea*), spadefoot toad (*Pelobates fuscus*), common and green toad (*Bufo bufo*, *B. viridis*), agile frog (*Rana dalmatina*), European common frog (*Rana temporaria*) and edible frog species group (*Rana esculenta* complex) can be found. This is the first published flatland occurrence of European common frog. Based on our own experiences, moorfrog (*Rana arvalis*) and common toad seem to be the most common. We could not observe any newts, European common frogs and common tree frogs, however, We did not conducted surveys directly on amphibious species. As for reptiles, we observed grass snake (*Natrix natrix*) and European pond turtle (*Emys orbicularis*) on one or two occasions. Stollmayer & *al.* [180] gives a thorough overview about the bird fauna of the territory, however, we could not observe significant presence of water fowls on the examined section of the stream.

Between 1991 and 2002 we continuously collected data and observations about the invertebrate fauna of the examined section of stream. The most profound general zoologic exploration took place during the series of examinations in 2002. At that time, from 15th March to 27th October (beside the ordinary Heteroptera sampling) we also conducted regular – fortnightly done – zoocoenological sampling, which consisted of identical methods during the full length of the examination period, namely silt sampling, water netting, surface netting, grass-netting of above-surface vegetation and plankton-net

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sampling. Before the sampling process we did frog count. Our examinations only extended to the waterbed and the immediate above-surface part of the vegatation – we wilfully avoided the bench line, during grass-netting just plants leaning over the riverbed from a 10 cm wide zone of the coastal line could be incorporated. We considered the examination of the vegetation stretching over the waterbed very important for four reasons. At first, because certain members of the semiaquatic predator guild (e.g. *Hydrometra* spp., semiaquatic wolf spiders etc.) and just hatched adults of aquatic insects are very often located here. Secondly, because one of the main kind of food of the *Gerris* species are „land" insects accidentally fallen onto the water surface. Thirdly, because the hereby abiding net-spinning spiders are important predators of the insects flying out of the water (e.g. gnats, mayflies), and finally because the herbivore insects can exert a significant effect on the community by eating on aquatic plants.

A summarising overview on the main data of general, zoological exploration in year 2002 are shown in *Table 1*.

A summarising overview of the main data of the general zoological survey conducted in the spring of 2002 can be seen in *Table 1*. Animals are classified into morphones because taxa in themselves are less explanatory from the aspect of seasonal dynamic patterns. It can be clearly seen from the table, that the different development stages or body size categories of the same taxon show fundamentally different coenological behaviour. To achieve a uniform structure, we followed the system of Papp [138] during higher taxonomical classification, and we took the work of Móczár [118] as a base for naming morphones (at levels below families) even when we used different works for identification. Samples from the water body are given conjugated in order to achieve briefer data and transparency, because the method of collection can be generally presumed from the name of the taxon (and body size category). Numerical data given in the table can be compared row-wise, since comparison between different rows can be informative only in case of identical body-size categories.

Methods of field monitoring

We have been studying the Heteroptera community of the Szilas stream since 1991. In this study, the field data collected in 1996 are processed and evaluated. The seasonal dynamic patterns in the vegetation period (between March and November) with the greatest frequency were observed in 1994 (monthly), in 1996 (weekly) and in 2002 (fortnightly). We only collected qualitative data in 1991 and 1992. In 1993, 1995, 1998 and 1999 we sampled 3-4 times in the vegetation period. From the years of 1997, 2000 and 2001 we do not have any data. We used a method, which was designed for qualitative purposes – however being rather semi-quantitative – to explore the seasonal-dynamic patterns. In the winter period we only conducted qualitative examinations.

Based on our data and observations it can be stated that in the period between 1991 and 2002 on the examined section of Szilas stream (under Lake Naplás) fundamental changes have not been occurred nor in the hydrological and vegetation characteristics, nor in the structure of the Heteroptera community.

For the purpose of our studies, we chose a 300 m long section of Szilas stream below the Naplás Lake. The peculiar section was chosen for its homogeneity from floral and hydrological aspects.

The coenological survey of Heteroptera consisted of the simultaneous application of two very different methods, namely a quantitative area-closing sampling and a roving hand-webbing collection.

Table 1: *Conjugated data of the fauna exploration in 2002. Italic names are so-called morphons, which had been conceived from some kind of taxonomic name and body size category (I: below 5 mm, II: between 5–10 mm, III: above 10 mm) or in case of insects, from morphological state (larva, pupa, adult etc). Table contains data from aquatic samples (typed in bold) and data gained from grass-netting the above-surface vegetation (typed in normal characters).*

Morphons	**Sampling period (pairs of months)**			
	III–IV	**V–VI**	**VII–VIII**	**IX–X**
CNIDARIA				
Hydrozoa				
Hydra circumcincta I	312	35	26	8
PLATYHELMINTES				
Turbellaria				
Planaria (Dugesia) lugubris I	8	20	9	5
Planaria (Dugesia) lugubris II	2	16	42	10
Planaria (Dugesia) lugubris III	2	4	10	13
Policolis nigra I	5	6	26	4
Policolis nigra II	23	26	34	
Policolis nigra III	1	1	2	
MOLLUSCA				
Gastropoda				
Eucolonus fulvus I		17	46	50
Euconulus fulvus II		2		
Euconulus fulvus II	85	149	16	8
Euconulus fulvus III		2	1	
Euconulus fulvus III		15	5	
Granaria frumentum I			1	
Granaria frumentum II			2	14
Helicopsis striata I				
Helicopsis striata II		1		
Helicopsis striata III		1		
Lymnea palustris II	1	1	2	
Lymnea palustris III	3	1	2	
Lymnea truncata II			1	
Succinea elegans I	19	8	41	91
Succinea elegans II	14	74	7	16
Succinea elegans III		4		
Succinea I			1	1
Succinea II	3	7	11	10
Zonitoides nitidus I		1		
Zonitoides nitidus II	2			1
Bivalvia				
Pisidium hibernicum I	24	23	193	500
Table 1. continued from page 10				
Pisidium obtusale I	2		2	13
ANNELIDA				
Clitellata				
Oligochaeta				
Eiseniella tetraedra III		14		1
Tubificidae (Potamothrix) III	2285	4282	2485	4945
Hirundinea				
Erpobdella octoculata I			20	5
Erpobdella octoculata II	12	3	6	7

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Morphons	Sampling period (pairs of months)			
	III–IV	V–VI	VII–VIII	IX–X
Hirundinea (*continued*)				
Erpobdella octoculata III	12	23	22	24
Glossiphonia complanata II	1			
Glossiphonia complanata III	1			1
ARTHROPODA				
Arachnida				
Araneae				
Agriopidae I			2	
Agriopidae I		1	5	7
Dictynidae: Dictyna arundinacea II				1
Lycosidae I	3	5	18	2
Lycosidae II	5		6	
Lycosidae: Pardosa amentata I	1			
Lycosidae: Pirata latinans I		6	1	6
Lycosidae: Pirata piraticus I	3	1	52	14
Lycosidae: Pirata piraticus II	2	6	1	24
Lycosidae: Pirata piraticus III		1		
Pisauridae I	5		15	3
Pisauridae II	1	1	2	4
Salticidae II				1
Tetragnathidae (T. extensa) I	10	2	6	21
Tetragnathidae (T. extensa) II		7	15	2
Tetragnathidae (T. extensa) III				2
Tetragnathidae I	71	31	30	47
Tetragnathidae II	2	27	26	6
Theridae I			1	
Theridae II		1		
Thomisidae I		1		
Thomisidae I	21	3	12	13
Acari				
Hydracarina I	3	4	1	3
Ixodes ricinus I	3			
Ixodeus ricinus I	1			
Oribatida I	17	25	22	8
Malacostraca				
Edriophthalma				
Asellus aquaticus I	26	6	1	
Asellus aquaticus II	40	5	10	1
Gammarus roeseli I	1591	3977	5617	7536
Gammarus roeseli II	1473	6445	5073	6340
Gammarus roeseli III	525	2007	2000	2950
Oniscoidea I	1			
Oniscoidea II	1	2		1
Maxillopoda				
Copepoda				
Acantocyclops (?) I	26	1		
Acantocyclops robostus I	317	1769	1983	1862
Eucyclops I		28	133	34
Eudiaptomus I	14			330
Ostracoda				
Heterocypris salina I	3	11	3	
Limnocythere sanctipatricii I	28	37	536	26

Morphons	Sampling period (pairs of months)			
	III–IV	V–VI	VII–VIII	IX–X
Phyllopoda				
Cladocera				
Chydorus sphaericus I		4	384	7
Parainsecta				
Collembola				
Entomobrya quinquelineata I		3	2	3
Lepidocyrtus cyaneus I		2	10	7
Lepidocyrtus paradoxus I		2	4	3
Orchesella cincta I				1
Podura aquatica I	28	28	10	55
Sminthurides aquaticus I				2
Insecta				
Ephemeroptera				
Baetis I larva	331	216	786	87
Baetis II larva	131	227	1622	269
Baetis II adult		2		
Baetis III larva		75		
Cloeon larva		18		4
Prosopistoma I subimago				1
	III-IV.	V-VI	VII-VIII	IX-X.
Odonata				
Aeschnidae:Aeschna affinis III larva			2	1
Agrionidae: Ischnura elegans III adult		2		
Agrionidae: Ischnura pumilio III larva		3	6	
Agrionidae: Ischnura pumilo I larva			10	1
Agrionidae: Ischnura pumilo II larva	1	1	99	85
Agrionidae: Ischnura pumilo III larva	12	23		168
Agrionidae: Coenagrion puella III adult		1		
Agrionidae: Ischnura elegans III larva		2		2
Agrionidae: Platycnemis pennipes I larva			77	
Agrionidae: Platycnemis pennipes II larva			18	3
Agrionidae: Platycnemis pennipes III larva		3	2	7
Agrionidae: Platycnemis pennipes III larva			1	
Agrionidae: Pyrrhosma nymphula III adult		1		
Calopterigidae: Calopteryx splendels III adult		1		
Calopterigidae: Calopteryx splendens II larva	1			
Calopterigidae: Calopteryx splendens III larva	4			1
Libellulidae: Libellula fulva II larva				2
Libellulidae: Libellula quadrimaculata I larva		1		1
Libellulidae: Libellula quadrimaculata II larva		1		1
Libellulidae: Libellula quadrimaculata III larva	2	2	2	5
Libellulidae: Orthetrum brunneum II larva			2	
Libellulidae: Orthetrum brunneum III larva			1	
Libellulidae: Orthetrum coerulescens III larva	6	2		
Libellulidae: Orthetrum coerulescens III larva			1	
Plecoptera				
Nemouridae: Nemoura cinerea II adult	108			
Nemouridae: Nemoura II larva	258			
Ensifera				
Conocephalidae: Conocephalus fuscus IIIadult			18	
Ephippigeridae: Ephippigera ephippiger IIIadult		3		
Caelifera				
Acrididae III adult		3		
Acrididae: Chorthippus parallelus III adult			18	

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Morphons	Sampling period (pairs of months)			
	III–IV	V–VI	VII–VIII	IX–X
Thysanoptera				
Bolothrips cingulatus I adult	12		2	
Limothrips angulicornis I adult				3
Thrips phsapus I adult			2	
Thysanoptera I adult		4	1	
Heteroptera				
Anthocoridae: Orius laticollis I adult	1		1	
Corixidae: Hesperocorixa linnaei I larva	1			
Corixidae: Hesperocorixa linnaei I adult	1			
Corixidae: Micronecta meridionalis I larva	5	7	2	
Corixidae: Micronecta meridionalis I adult			2	
Corixidae: Sigara lateralis I adult			2	
Corixidae: Sigara striata I adult		1		
Gerridae: Aquarius paludum III adult	1	1	2	1
Gerridae: Gerris argentatus I adult		3		
Gerridae: Gerris asper II adult	1			
Gerridae: Gerris lacustris II adult	66	87	346	254
Gerridae: Gerris lacustris larva		156	784	352
Gerridae: Gerris odontogaster II adult	1	2		
Gerridae: Gerris odontogaster larva		1		
Gerridae: Gerris thoracicus adult	2	4	5	
Hydrometridae: Hydrometra stagnorum II larva		2	7	
Hydrometridae: Hydrometra stagnorum III adult	2	12	13	5
Lygaeidae: Cymus glandicolor I adult	1			
Lygaeidae: Cymus melanocephalus I adult		1		
Lygaeidae: Raglius confusus I adult			1	
Miridae: Adelphocoris lineolatus I adult			2	
Miridae: Adelphocoris seticornis I adult			1	
Miridae: Polymerus holosericeus I adult			2	
Miridae: Stenodema calcarata I adult	2			
Nabidae: Nabis ferus I adult			2	1
Nepidae: Nepa cinerea II larva		27	62	1
Nepidae: Nepa cinerea III adult			5	9
Notonectidae: Notonecta glauca II adult		3	18	48
Notonectidae: Notonecta glauca II larva		7	7	
Pleidae: Plea minutissima I adult		4		
Saldidae: Saldula opacula I adult			1	
Scutelleridae: Eurygaster maura II adult		2		
Tingidae: Agramma confusum I adult			1	
Tingidae: Dictyla hamuli I adult	1			
Veliidae: Microvelia reticulata I adult				2
Auchenorrhyncha				
Cercopidae, Lepyronia II adult		1		
Cercopidae, Philaenus spumarius I adult			1	
Cercopidae, Philaenus spumarius II adult				1
Cercopidae: Aphrophora alni II adult				1
Cicadellidae : Cicadella II adult		1	2	3
***Cicadellidae : Cicadula sp II** adult*			1	4
Cicadellidae I adult	6	19	37	60
Cicadellidae II adult		8	22	12
Cicadellidae, Edwardsiana rosae I adult				1
Cicadellidae, Eupteryx urticae II adult				2
Cicadinea I larva		5	4	2
Delphacidae I adult		4	8	37

Morphons	Sampling period (pairs of months)			
	III–IV	V–VI	VII–VIII	IX–X
Auchenorrhyncha (*continued*)				
Delphacidae II adult		11	5	1
Delphacidae: Calligypona sp I adult				1
Delphacidae, Dicranotropis II adult			1	
Delphacidae, Kelisia guttala I adult				6
Issidae I adult				2
Sternorrhyncha				
*Aphidinea I *A* adult*		9	17	60
*Aphidinea I *B* adult*			3	27
Aphidinea I adult		16	30	139
Psyllinea I (Aphalara calthae) adult			1	
Coleoptera				
Anobiidae I adult				1
Anthicidae I adult			1	
Anthribidae I adult		1	7	1
Bruchidae I adult			4	
Byrrhidae I adult			1	
Cantharidae I adult		2		
Carabidae I adult				1
Carabidae II adult		1		
Cerambicidae I adult			1	
Cerambicidae III adult		1		
Cerambycidae I adult	7			
Cerambycidae II adult			2	
Cerambycidae III adult		1		
Chrysomelidae, Donacia semicuprea I adult	3	16	2	3
Chrysomelidae I adult		8	7	
Chrysomelidae II adult		173	2	2
Coccinellidae I adult		1	8	
Coccinellidae I adult	1	8	2	
Coccinellidae II adult			1	
Coccinellidae II adult			2	
Curculionidae I adult		3	2	4
Curculionidae I adult	8	2	10	2
Curculionidae II adult			2	
Curculionidae II adult	4	8		
Curculionidae III adult			1	
Drilidae I adult		1		
Dytiscidae I adult		1		1
Dytiscidae I adult		3		1
Dytiscidae III adult		1		4
Dytiscidae, III larva	1		22	15
Gyrinidae II adult		5	4	
Haliplidae I adult		1	6	2
Helophoridae I adult	2	6	3	4
Helophoridae I larva		7		
Hydrophilidae I adult	6	11	114	13
Hydrophilidae II adult		1	1	
Hydrophilidae III adult			1	
Hydrophilidae/+Helophoridae/ I adult	6	2	6	
Lampyridae II adult		1		
Lampyridae III adult		1		
Lathridiidae I adult		1		
Malachiidae II adult			1	1

Morphons	Sampling period (pairs of months)			
	III–IV	V–VI	VII–VIII	IX–X
Coleoptera (*continued*)				
Mordellidae I adult		1		
Mordellidae I adult			1	
Mordellidae II adult		2		
Mycetophagidae I adult			2	
Oedemeridae II adult			1	
Scarabaeidae I adult	1	3	1	
Scolytidae I adult		1		
Spercheidae I adult			3	
Staphylinidae I adult		2	3	2
Staphylinidae I adult		1	2	1
Megaloptera				
Syalidae: Sialis fuliginosa III, adult	2			
Syalidae: Sialis flavilatera I larva			1	
Syalidae: Sialis flavilatera III adult	1			
Hymenoptera				
Agriotypidae II adult	1			
Andreidae III adult			1	
Aphidiidae I adult		1	2	
Apidae III adult			1	
Apoidae III adult		1		
Aulacidae I adult		1		
Belytidae I adult			1	
Ceraphronidae I adult		1		
Eulophidae I adult			1	
Formicidae I adult		37	50	10
Formicidae I adult	7	43	52	3
Formicidae II adult		1	2	
Formicidae II adult			2	
Gasteruptonidae II adult			2	
Hybryzonidae I adult			1	
Ichneumonidae I adult			1	
Ichneumonidae I adult		1	12	16
Ichneumonidae II adult	1	6	8	2
Ichneumonidae III adult			1	
Maumaridae I adult		1		
Orussidae I adult				1
Platygasteridae I adult			1	
Scelionidae I adult				2
Tenthredinidae I larva		1	1	
Tenthredinidae II adult			1	
Tenthredinidae I adult				3
Tenthredinidae II adult	2	1	1	
Tenthredinidae III adult		5		
Trichoptera				
Hydroptilidae: Ptilocolepus granulatus I larva	2			
Leptoceridae: Athrispodes aterrimus larva			2	
Limnephilidae: Grammotaulius III adult			3	
Limnephilidae: Limnephilus II larva	340	115	10	
Limnephilidae: Limnephilus III adult			8	12
Limnephilidae: Limnephilus III larva	667	423	111	
Phryganeidae: Oligostomis III adult			9	

Morphons	Sampling period (pairs of months)			
	III–IV	V–VI	VII–VIII	IX–X
Lepidoptera				
Noctuidae I adult		1	2	1
Noctuidae II adult		4	2	
Noctuidae III adult		1	3	1
Pyralidae I larva		1	1	
Diptera				
Agriotypidae II adult	2			
Agromyzidae I adult			1	1
Annisopodidae II adult	3			
Anthomyzidae I adult		2		
Anthomyzidae I adult			4	
Asilidae I adult	12			
Asilidae I adult		1		
Asilidae II adult		1		
Asilidae I larva		2		
Asteiidae I adult			1	
Camillidae I adult	4	5	11	1
Cecidomyiidae I adult		1	1	2
Cecidomyiidae I adult	6			1
Ceratopogonidae I adult	2	23	25	
Ceratopogonidae II adult				2
Ceratopogonidae I larva	47	33	4	25
Ceratopogonidae II larva		9		
Chironomidae (a) I adult	5	2	3	25
Chironomidae (a) I larva	243	1144	3464	1117
Chironomidae (a) I pupa	11		60	67
Chironomidae (b) II larva	55	43	130	116
Chironomidae I adult	14	3	20	151
Chloropidae I adult	68	19	173	22
Choloropidae I adult	1		10	9
Conopidae III adult	2			
Culicidae I adult	1		2	9
Culicidae II adult				2
Culicidae II adult		1	1	
Culicidae I larva		33	136	6
Culicidae II larva	1	10	87	4
Culicidae II pupa		3	10	6
Curtonotidae I adult			1	
Dixidae II adult				2
Dixidae I adult			1	
Dixidae I larva			2	4
Dolichopodidae I adult			3	1
Dolichopodidae II adult		1		
Dolichopodidae II larva		2	4	
Drosophilidae I adult		1	2	
Dryomyzidae II adult			1	
Empididae I larva			2	6
Empididae II larva	4			2
Empididae II adult		3	2	
Empididae III adult	1			
Empididae III adult	3			
Ephydridae I adult				55
Ephydridae I larva			6	
Ephydridae I adult	10	17	10	
Helomyzidae I adult			1	1

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Morphons	Sampling period (pairs of months)			
	III–IV	V–VI	VII–VIII	IX–X
Diptera (*continued*)				
Helomyzidae II adult	7			2
Lauxaniidae II adult		16	1	9
Limnoiidae II adult	4	4	4	1
Limonidae I adult			2	
Limonidae II adult				1
Lonchopteridae II adult	7	5	8	5
Megamerinidae II adult			5	
Milichiidae II adult	8		1	5
Muscidae I adult		6		
Muscidae I pupa		2		
Musicidae I adult			2	
Musicidae II adult		1	14	5
Mycetophilidae I adult	9			
Odiniidae I adult		5		
Opomyzidae I adult			4	1
Otididae II adult		1		
Pallopteridae I adult			2	
Periscelidae I adult			3	
Phoridae I adult			2	
Pipunculidae I adult			3	
Pipunculidae II adult		1		
Pipunculidae II adult		1	1	
Platypezidae I adult		2	5	2
Ptychopteridae II adult		4	5	
Rhagionidae II adult		5		1
Rhagodidae I larva			5	
Rhagodidae II larva		1	2	
Rhagodidae III larva			1	
Scatopsidae I adult				2
Sciaridae I adult	2	3	1	
Sciaridae II adult		1		
Sciaridae II adult		1	2	
Sciomyzidae I adult		2	4	
Sciomyzidae II adult			7	
Sciomyzidae II adult			1	
Sepsidae I adult			6	
Sepsidae II adult	3			7
Sepsidae II adult			1	
Simulidae I larva			3	4
Simulidae II larva	14		10	1
Simulidae II pupa	1			
Sphaeroceridae I adult				2
Sphaeroceridae I pupa	4	3		
Sphaeroceridae I adult			3	2
Stratiomydae II adult		2	2	
Stratiomydae I larva	2	2	5	4
Stratiomydae II larva	2	3		2
Stratiomydae III larva			1	
Syrphidae II adult	6	3	6	2
Syrphidae III adult		3		2
Syrphidae III adult				1
Syrphidae I larva		2		
Tabanidae I larva		1		
Tabanidae II larva			1	

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Morphons	Sampling period (pairs of months)			
	III–IV	V–VI	VII–VIII	IX–X
Diptera (*continued*)				
Tabanidae III larva	1	1	1	3
Tachinidae II adult	4	2	4	
Thaumaleidae I adult	1			
Tipulidae III adult	1	4	3	2
Ulidiidae I adult			2	
VERTEBRATA				
Amphibia				
Anura				
Rana arvalis I egg		538		
Rana arvalis I tadpole		104		
Rana arvalis III tadpole			21	
Frogs (by count)	64	53	76	25

For the purpose of area-closing sampling we used two pieces of 5 m long curtain webs, which had on its lower part a canvas-covered iron chain and, on its top, a stretching rope. By the application of the two curtain webs and some tapered stakes, an optional part of the stream (in the range of 2–10 m long sections) can be closed unpassably for Heteroptera. The curtain web does not hampers the water current and from the separated section Heteroptera can be collected with hand-web. Length, width and areal distribution of parts with different depth can be recorded from the separated section. Furthermore, it is very important to record the vegetation coverage of the closed area.

The data gained can be applied for stream length, area, water volume and these data can be even applied for derivative data weighted by vegetation parameters. These data can be standardized by using these parameters. In this work we used our data scaled to 10 m stream-length, without any width, depth or vegetation correction. This sampling method has – apart from its numerous advantages – some disadvantages as well, and the majority of these disadvantages originates from the fact, that the gained sample can not be divided into subsamples, and even the separation of more, lesser sections is not advisable because of the rapidly growing fringe effect. In order to eliminate these disadvantages, we conducted roving hand-webbing collection on the full length of the section. One objective of this is to test the representativeness of the closed section sample for the full length of the observed area (verification of spatial inhomogeneities belonging to a given scale-level). The other objective is to gain an other, a faunistically more complete species list which contains even the rarer species in greater proportion. When during the roving examination some doubts emerged concerning the representativity of the sample, we repeated the area-closing on an other section and compared the two quantitative samples. Such a case happened only twice during the 10-year long examination period and besides, these proved to be undue. (In the case of a more inhomogeneous coastal section, the desired extent of representativity could be achieved only by averaging more, layered samples).

In our present study, we have not exploited the quantitative characteristic of the samples (absolute individual density estimation), we only used data to compare identical areas at different dates (semi-quantitative feature). To enhance the reliability of the data we applied logarithmic transformation (to outline different levels of magnitude), thus our data are comparable with the results of other authors' surveys conducted even by different methods.

Methods of simulation modelling

The presently used simulation modelling methods applied for seasonal dynamics of population collectives have been developed during our earlier works together with our students and colleagues [60, 61, 96, 97, 98, 99]. This methodological approach serves the purpose to make monitoring and simulation methods easily comparable to each other. To reach this goal, we applied temporally discrete (daily recorded) deterministic biomass-growth simulation equations and fenological connecting-functions based on them. The most detailed summary and general description of the model system can be found in our paper: [98]. The biomass module of the general model system consists of a temperature-dependent inner reproduction rate and other factors simulating interpopulational interactions and predational factors. The latter factors summarise effects of the in- and out-running edges of the interaction graph in such a manner, that the density-dependency, all the two ways of consuming interactions (who consumes and who is consumed), static and dynamic preference, real and apparent competition can also be regarded.

In our present study we used a strongly simplified version of the above-mentioned model system which has been applied for the examined situation. In this, the interactions of the populations only emerge involved in the temperature-dependency. Simulation calculations could be done easily in MS Excel tables, the starting values of parameters has been defined based upon our field experiences and publications listed in the literature overview section. The paper from Bacchi et al [8] made us to realize the opportunity of radical simplification of the models. This paper contains faunistical data about aquatic and semiaquatic Heteroptera in Italian habitats, and the appearance data are also illustrated on pH and temperature charts. These graphs show that the effect curve of temperature does not come up with only one optimum, – as we supposed in our former paper [98], but it can – supposedly because of population interactions – also be multi-peaked. Correction of the starting values defined by estimation (thus the fitting of the model to field data) has been done with minimizing the sum of squares (method of least squares) with the help of MS Excel Solver program.

Methods of coenological pattern-evaluation

Examination of coenological and ecological data is a quite complicated multivariate problem, which can be handled only with the tools of biomathemathics and informatics. In case of considering more than three coenological variables (taxon or morphon) we need multivariate data-structure explorative methods to evaluate coenological patterns. The most important methods of a multivariate data analysis are the divisional (classification) and dimension-reducing (ordination) processes. About the traditional methods of multivariate biological data evaluation, works of Podani [143, 144, 145, 146] give detailed overview and outstanding methodological help. Concerning classification, work of Blashfield & Aldenderfer [26] gives a good starting point.

Methodological development results (related to state-planes) used in this work can be found in our former publications. The basic idea for these appeared first in our papers [72, 73], which are dealing with water qualification based on behaviour and features of Heteroptera and habitat characterization, however, at that time the introduction of indicator coordinates took place by fuzzy clustering and not by ordination. The indirect ordination methodology used in this paper has been developed continuously in publications [48, 49, 50, 70, 79, 80] and was applied in its current form in the paper [98]. Some development of the method group were described in publications [155, 156].

The third pillar of the present work includes our biomathematical developments which are connected to simulation models [43, 60, 61, 96, 97, 98, 99].

The more and more exact description of states and conditions and the exploration of connections can be successfully carried out, the greater, the detailed, the more exact databases can be created. However, the greater the database is (also from the aspect of variables and objects) the more difficult is to overview the phenomena it represents. A key element therefore is the decent application and use of dimension-reducing methods. Classification methods are suitable to control the results of ordinations and they are also of a great help in further navigation in the gained, reduced dimension patterns.

The application of generally known and used ordination methods is often significantly hampered in case of huge quantity of data, especially if our objective is the simultaneous use of monitoring and simulation data.

Emerging problems can be summarized as follows:

1. Limited capacity of dimension-reducing methods. By the growth of data matrices, the running time also grows excessively, and also by the application of softwares and computers regarded as currently the best, the capacity greatly lags behind the quantity of data which would be ideally used.
2. The running problems of the majority of well-applicable programs, which is of no trouble by independent evaluations, but they make harder the evaluation of greater databases in parts.
3. The problem of newer objects. At the beginning of data evaluation, we do not possess all pieces of information which are necessary later, because the point of monitoring is that it provides a continuous observation system. On the other part, if we need to reapply all the former data by every new casual evaluation, it creates many redundancies, which in addition endangers even the stability of formerly defined considering points. The reason for this is that ordination methods always look for the actual similarity patterns of the examined objects, and all new objects can exert its effect on this process.
4. Tightly correlates with the above-mentioned facts the problem of the reliability of data, because data which are considered by us as highly or less reliable, influence the result of ordination in the same extent. Thus, incorrect data not only causes problem in the position of the given object which contains it, but can also influence the whole pattern, which obviously can not be tolerated.
5. Another problem is the deformity of the sampling net itself, because if there are certain object types that are overrepresented compared to others, then the significance of value combinations contained in more prevalent type of objects becomes overestimated and this can severely endanger the consequent interpretation of the results.
6. Sometimes the examination of new (or derived) variables, is necessary, which do not influence the pattern itself, but they behaviour inside the pattern can be informative.
7. And finally, if we even disregard the above-mentioned facts, there would be the problem that the huge quantity of objects (even in the reduced dimension space or plane) will result in an extremely dense cloud of points, so the possibility of alternative display must be provided even inside the same pattern.

To eliminate all these problems we developed the methodology of stable state-planes which are based on indirect ordination [48, 49, 50, 72, 79, 80].

The primary objective of our work has been to develop a unified data-handling and state-evaluation methodology for the temporal-spatial modelling and temporal-spatial simulation modelling of the ecosystems. The state-plane systems developed by us can be of great help to the examination of the following types of problems:

- comparison of ecosystem-models which simulate in time and space with real data, the optimal setting of the fitting parameters of the models, and testing of models;
- informatic handling and evaluation of data of ecologic monitoring;
- direct application of modelling results in the monitoring;
- unified handling of field trial data and facilitation of statistical analyses;
- simulation of effects of experiments;
- examination of possible effects of climatic change;
- support of the methodology of ecological risk assessment.

The essence of indirect ordination method can be summarized as follows:

The direct dimension-reducing step made by traditional multivariate pattern analysis (data structure explorative) method algorithms (later referred as direct ordination) is not carried out on the objects, but – due to the principle of attribute-duality – on the variables. Thus, it can be considered as a kind of direct ordination, which explores the similarity pattern of the original variables (as objects) based on a part of original objects (as variables) – analysis of a transposed matrix.

Ordination of the real objects is carried out indirectly, in a separate step, using the coordinates of the variables. The simplest way of this could be if we choose for the coordinates of the object in the indirect ordination some kind of function (average, weighted average, sum etc.). Thus, the objects in this step are going to be displayed on the graph independently of their relative positions, this way solving some part of the problems mentioned among the objectives.

Direct ordination carried out to define the coordinates of variables does not apply to the whole series of data, but only to a selected, strongly filtered “reference database”, which we have the opportunity to overview and know perfectly. Only this reference database has to represent the real relations between variables, later incoming data will not be able to influence this. Thus, the reliability problem becomes solved as well.

In case we calculated the coordinates of the objects, the indirect ordination is theoretically determined, but for its comprehensibility and multifunctional use, the functions of alternative display and graphics must also be provided. These functions are provided by GIS which combines the methods of database-handling and graphic display – solving the task mentioned in the last point of the objectives.

Steps of building state-plane systems can be seen in *Fig. 1*.

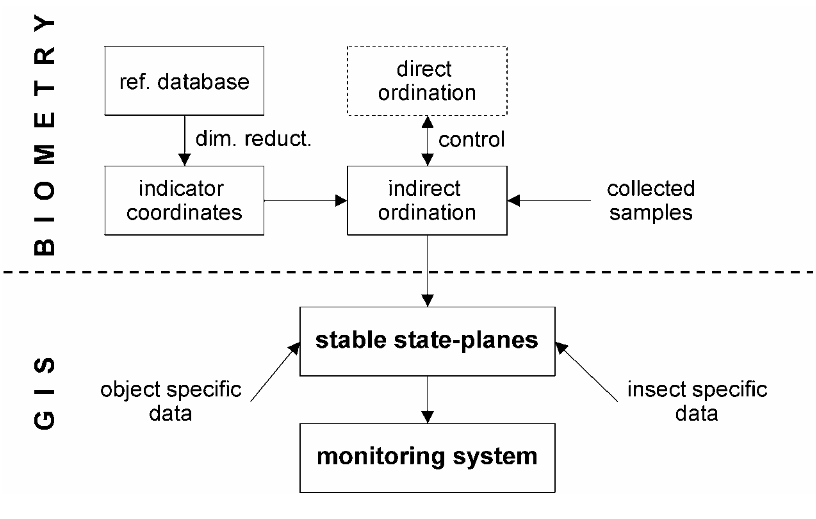

Figure 1. *The process of building state-planes*

On the chart, going from the left to the right and from the top to the bottom can we proceed from the start to the use of the system. First step is the construction of the reference database. All the variables must be involved into the database which are regarded as state indicators in respect of comparison of the objects. It is very important that these indicators would be interpretable in connection with each possible objects and could take only one value in each case. Variables must be measurable independently of each other, so they can not be values computed from each other. However, from the aspect of the goodness of dimension-reduction it is of great advantage, that these variables should be in the closest statistical relationship with each other, because this way they represent information about essential characteristic (state) of the observed system. Variables omitted from the reference database and their derivatives become examinable later and with different methods with the help of the state-plane.

Next step is the direct dimension reduction based on the reference database. Its aim is to determine the coordinates of the indicator variables. When it is accomplished, a functional connection must be defined with the help of indicator coordinates between the actual values of the variables used to characterize the objects and the object coordinates of indirect ordination. If the indirect ordination is at hand, there is a possibility to check, how much the original similarity pattern of the objects would be damaged during the display with indirect ordination (for this purpose, the Shepard-diagram which is usable by non-metric multidimensional scaling [NMDS] can also be used). It could also be very helpful to judge the goodness of indirect ordination if the direct ordination of a part of the examined objects is carried out and this can be compared with the similarity pattern achieved from the indirect ordination mentioned above. Following these testings, the behaviour of additional objects and variables (or even derived indicators) can be examind with the help of the stable (because it is interpreted between fixed points) state-plane. By the alternative display of different objects and variables on the state-plane the state-plane can be mapped, based on different aspects of examination. Among others, GIS is also suitable for handling these alternative “thematic state-maps”. The state-plane system is suitable for the purpose of monitoring and evaluating infinite objects until the state of the objects remains between the states given by the reference database. Would appear there a basicly new phenomenon, it is quite uncertain that its significance would be displayed decently on the state plane. This fact calls the attention again to the significance of constructing the proper reference database. This problem can be solved – in case it is needed – with the construction of many different state-planes – according to the objective of the examination – and handling (even like meta-analyses) these alternative state-planes in one unified system.

Multivariate, stable state-plane systems based on indirect ordination are suitable for the solution of the problems defined in the objectives. These state-plane systems are however not only suitable for the mechanical display of data-series resulting from monitoring and simulation or to achieve objective comparability, but they can also fundamentally integrate these two methodologies, which came across different paths during the history of science. In case of successful fitting of these simulation models on real monitoring data-series, then it should open a broader than before horizon of interpretability of results and conclusions. In this case, there is a possibility to construct reference databases partly from empirical and partly from simulated data, which means that state-planes can be ready to handle extraordinary situations never experienced before, and this could render the usefulness and applicability of state-plane systems quite general.

Terminology, definition of concepts used

Systems of professional terms used in supraindividual biology (meaning of concepts and the relations between them) are strongly tied to the different schools and theoretical trends. Thus, the very same professional term could bear totally distant meaning in different works. Since concepts and terms are used in many ways by different authors, it seemed to be inevitable to clearly define the terminology applied in this work.

However the objective of the definitions listed here should not be considered as a general formulation. Our task can not be therefore the modification or restriction of the well-known meanings of individual concepts. Nor could it be the definition of them by scientific claims (because many had done this before our work in many ways), only to make the terminology of this current work clear (in order to facilitate the independent comprehensibility of different parts of the text), so we regard it valid only in this respect.

- **Coenology**: A discipline belonging to supraindividual (syn-) biology, which deals with the description of coexistent states of living beings' (in this case animal) communities. (Pragmatic streams of this discipline are often referred to as community ecology).
- **Community structural (or coenological) state**: The coenological state of an examined object (habitat, sampling place, sampling unit etc.) can be explained if the presence (mass indicator, measurable with a preliminarily set observation method) or absence of the living objects involved in the study (actually defined as variables) are given. Thus, the assignment of coenological state means the disclosement of a species list and/or a quantified species list (perhaps density distribution) by very strict rules.
- **(Coenological) change of state**: By the terminology of this current work, every temporal-spatial processes, in which any of the variables of the above-mentioned coenological state alters, is regarded as a (coenological) change of state, regardless to its reason or statistical feature.
- **Coenological behaviour**: Temporal or spatial pattern of change of state, which can apply for the whole or for any part of the community.
- **Coenological monitoring or monitoring**: Series of field observations done in many steps with defined objective and adequate method, which traces temporal changes of state on a fixed scale and period of time.
- **Monitoring system**: Monitoring is only feasible in the frame of a monitoring system. A monitoring system can be considered defined, when the following necessary conditions are set: objects (or the group of objects), variables (in this case: living beings), period of examination, frequency (or other succession) of the observed (sampling) units, methods of data collection, method of filling of the database and methods of primary data processing. In this current work, only series of examinations conducted in the same year can be considered as monitoring.
- **Simulation**: Generation of artificial data series with the help of a mathematical model. Data structure resembles to temporal coenological change of state.
- **Simulation model**: Very precise formulation of hypotheses and conditions applying to a temporal change of state with mathematical methods. (When the system of hypotheses and conditions are put in text, it can be accomplished by many (even significantly different) simulation models, because model is a more accurate, therefore a more restricted phrase.)
- **Scenario**: Result of the simulation, which is an (realistic, but not definitely real) outcome of the given model and parameters of the observed state of change.

Scenario is not a forecast, it is only one of the alternative possibilities. Usually probability of outcome can not be attributed to scenarios (or only in form of "professional estimate").

- (**Coenological) indication**: A signal, in this case the information content of coenological state or change of state, which applies to the state or change in state of other conditions or variable groups. In this work, it occurs exclusively in relation to seasonal dynamics (actual position in an intra-annual trajectory of change of state). In this case, similarity pattern shown by sampling units based on a group of variables (indicator organisms) indicates the similarity pattern of the identical sampling units based on other variables. (Indication does not denote cause and effect connection).
- **Indicator organism**: In the terminology of this current work, it is an optional kind of living organisation which we involve in the group of variables, such playing a role in the coenological indication. (Thus, in itself it does not indicate anything, it is only one of the units of the indicators of the coenological state, which gives us an indication.)
- **Morphon**: Some kind of living organism, which can be unanimously identified and distinguished based on morphological characteristics. In this current work, it is a unit which contains taxon (taxonomical name), life-cycle state and/or body size category. The applied indicator organisms were identified as morphones. The reason, why the concept of morphon had to be introduced was that different development stages of a taxon often showed more different behaviour than similar development stages from other taxa.
- **Autochtone species**: Its full life-cycle takes place on the examined habitat.
- **Allochtone species**: Spends only a part of its life-cycle on the examined habitat.
- **Coenological state-plane, stable state-plane**: Plane – created by indirect application of multivariate dimension-reducing methods (on a reference database) – on which all the states can be definitely signed with only one point and every change of state with two points or a vector (map of the possible states). The attribute "stable" refers to its special characteristic: newly, later drawn points do not influence each other's position unlike by direct ordination methods.

Results

Overview of the examined aquatic and semiaquatic Heteroptera community from the aspect of field data

Comparison with the fauna of other aquatic biotopes, and the analysis of the similarity patterns of the aquatic and semiaquatic Heteroptera fauna of the Szilas stream are in the following papers: [37, 74, 183].

During the examination of the Heteroptera species community of the Szilas stream, all collected Heteroptera individuals from water or water-plants has been identified, disregarding how they entered this biotop. Thus 57 species from 16 families came into the sight of the examination. So not only those aquatic and semiaquatic Heteroptera species (Gerro- and Nepomorpha) which stand in the closer direction of the examination were studied, but even those Heteroptera, which life-conducts are in different extent tied to water or even species living on the land (but drifted into water by chance). Most of the publications about aquatic biotops do not contain these data, so in this respect there were not possible to compare these facts with them. Table 2. shows the collected species and in what extent they are tied to water.

Table 2. *Heteroptera species collected on Szilas stream between 1991–2002 and categories of their life-conduct from the aspect of water-tiedness. Aquatic: *****; semiaquatic: ****; coastal: ***, living close to water because of their characteristic feed-plant (or from other indirect reason): **, frequent in the proximity of water (as well): **

taxa	water-tiedness
GERROMORPHA	
Gerridae	
Aquarius p. paludum (Fabricius, 1794)	****
Gerris argentatus Schummel, 1832	****
Gerris asper (Fieber, 1860)	****
Gerris lacustris (Linnaeus, 1758)	****
Gerris odontogaster (Zetterstedt, 1828)	****
Gerris odontogaster larva	****
Gerris thoracicus Schummel, 1832	****
Limnoporus rufoscutellatus (Latreille, 1807)	****
Hydrometridae	
Hydrometra stagnorum (Linnaeus, 1758)	****
Mesoveliidae	
Mesovelia furcata Mulsant & Rey, 1852	****
Veliidae	
Microvelia reticulata (Burmeister, 1835)	****
Microvelia pygmea (Dufour, 1833)	****
Velia s. saulii Tamanini, 1947	****
NEPOMORPHA	
Nepidae	
Nepa cinerea Linnaeus, 1758	*****
Ranatra linearis (Linnaeus,1758)	*****
Notonectidae	
Notonecta g. glauca Linnaeus, 1758	*****
Notonecta viridis Delcourt, 1909	*****
Pleidae	
Plea m. minutissima Leach, 1817	*****
Corixidae	
Corixa affinis Leach, 1817	*****
Corixa panzeri Fieber, 1848	*****
Corixa punctata (Illiger, 1807)	*****
Hesperocorixa linnaei (Fieber, 1848)	*****
Paracorixa c. concinna (Fieber, 1848)	*****
Sigara falleni (Fieber, 1848)	*****
Sigara lateralis (Leach, 1817)	*****
Sigara n. nigrolineata (Fieber, 1848)	*****
Sigara striata (Linnaeus, 1758)	*****
Cymatia rogenhoferi (Fieber, 1864)	*****
Micronecta scholtzi (Fieber, 1860)	*****

taxa	water-tiedness
LEPTOPODOMORPHA	
Saldidae	
Chartoscirta elegantula (Fallén, 1807)	***
Chartoscirta cincta (Herrich-Schäffer, 1841)	***
Saldula arenicola (Scholtz, 1847)	***
Saldula pallipes (Fabricius, 1794)	***
Saldula pilosella (Thomson, 1871)	***
Saldula saltatoria (Linnaeus, 1758)	***
Saldula opacula (Zetterstedt, 1838)	***
CIMICOMORPHA	
Tingidae	
Dictyla humuli (Fabricius, 1794)	**
Agramma confusum (Puton, 1879)	
Tingis ampliata (Herrich-Schäffer, 1839)	
Nabidae	
Nabis ferus (Linnaeus, 1758)	*
Nabis pseudoferus Remane, 1949	
Nabis punctatus Costa, 1847	
Miridae	
Stenodema calcarata (Fallén, 1807)	*
Polymerus holosericeus (Hahn, 1831)	
Adelphocoris seticornis (Fabricius, 1775)	
Adelphocoris lineolatus (Goeze, 1778)	
Orthops sp.	
Anthocoridae	
Dysepicritus rufescens (Costa, 1843)	**
Orius laticollis (Reuter, 1884)	
Orius (Heterorius) sp.	
PENTATOMOMORPHA	
Scutelleridae	
Eurygaster testudinaria (Geoffroy, 1785)	
Eurygaster maura (Linnaeus, 1758)	
Pentatomidae	
Dolicoris baccarum (Linnaeus, 1758)	
Aelia acuminata (Linnaeus, 1758)	
Lygaeidae	
Cymus melanocephalus Fieber, 1861	*
Cymus glandicolor (Hahn, 1831)	*
Raglius confusus (Reuter, 1886)	

Summarized collection data of three observation years with the greatest temporal frequencies provides a possibility to examine the quantitative relations of the aquatic and semiaquatic Heteroptera communities (*Table 3*). Based on the data of *Table 3* it can be stated, that out of the 22 collected species only 6 are represented with greater than 1% dominance *(Gerris lacustris, G. thoracicus, G. odontogaster, Hydrometra stagnorum,*

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Table 3. *Relative occurrence by number of individuals of aquatic and semiaquatic Heteroptera (dominance%) on the examined section of Szilas stream*

Taxa	Szilas 1994	Szilas 1996	Szilas 2002
GERROMORPHA			
Gerridae			
Aquarius paludum paludum (Fabricius, 1794)		0.23	0.2
Gerris argentatus Schummel, 1832		0.05	0.13
Gerris asper (Fieber, 1860)		0.99	0.04
Gerris asper larva		0.49	
Gerris lacustris (Linnaeus, 1758)	**11.66**	**18.83**	**32.41**
Gerris lacustris larva	**10.76**	**24.64**	**55.65**
Gerris odontogaster (Zetterstedt, 1828)		**1.71**	0.13
Gerris odontogaster larva		0.28	0.04
Gerris thoracicus Schummel, 1832	0.27	**6.74**	0.47
Gerris thoracicus larva		**1.28**	
Limnoporus rufoscutellatus (Latreille, 1807)		0.1	
Limnoporus rufoscutellatus larva		0.01	
Hydrometridae			
Hydrometra stagnorum (Linnaeus, 1758)	**7.17**	**2.26**	**1.37**
Hydrometra stagnorum larva	0.63	0.53	0.4
Veliidae			
Microvelia reticulata (Burmeister, 1835)		0.03	0.09
Microvelia reticulata larva		0.04	
Velia saulii Tamanini, 1947 larva		0.01	
NEPOMORPHA			
Nepidae			
Nepa cinerea Linnaeus, 1758	**13.36**	**1.99**	0.59
Nepa cinerea larva	**34.26**	**4.12**	**3.86**
Ranatra linearis (Linnaeus,1758)		0.05	
Notonectidae			
Notonecta glauca glauca Linnaeus, 1758	**21.61**	**32.24**	**2.99**
Notonecta glauca larva	0.09	0.32	0.61
Notonecta viridis Delcourt, 1909		0.56	
Pleidae			
Plea minutissima minutissima Leach, 1817		0.55	0.17
Plea minutissima larva		0.1	
Corixidae			
Corixa panzeri Fieber, 1848		0.03	
Corixa punctata (Illiger, 1807)		0.26	
Hesperocorixa linnaei (Fieber, 1848)	0.18	0.19	0.04
Hesperocorixa linnaei larva			0.04
Sigara falleni (Fieber, 1848)		0.01	
Sigara lateralis (Leach, 1817)		0.44	0.07
Sigara lateralis larva		0.03	
Sigara nigrolineata nigrolineata (Fieber, 1848)		0.03	
Sigara striata (Linnaeus, 1758)		0.09	0.04
Micronecta scholtzi (Fieber, 1860)		0.73	0.07
Micronecta scholtzi larva		0.04	0.59

Nepa cinerea and Notonecta glauca). In *Table 3*, the nomenclature used by Aukema & Rieger [6] has been considered as a guide, and for the identification, works of Hufnagel & Vásárhelyi [76], Jansson [82, 83], Macan [108], Štusák [181], Vásárhelyi [188], Vásár-

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helyi & *al.* [195], Vepsäläinen & Krajewski [196] has been also considered. These six species together represent 95–99% of the individuals collected altogether. Thus, they are in dominant majority. From this six species, only four can be considered in its larva and adult form to be constant. Individual number of the collected larvae of *Gerris lacustris* and *Nepa cinerea* exceed the number of adults, thus these can be considered as autochtonic species. In the case of *Notonecta glauca* and *Hydrometra stagnorum* only a negligible number of larvae has been collected compared to the number of adults, so one part of the population is of autochtonic, the other is of allochtonic (migrant) origin. Individuals with ability to fly and with full wings are in minority among the collected individuals of *Gerris lacustris* (wing polymorphism), Notonects fly well, Nepas and Hydrometras are unable to fly. The low count of *Hydrometra* larvae can not be satisfyingly reasoned, although among Gerromorphans, most often hydrometrids crouch up to the plants above the water. The eight kind of living organisms (containing larvae and adults of the four constant-dominant species) were appointed as coenological indicator-organisms, their coenological indication (prediction) power is detailed in sections *Seasonal dynamic patterns represented by field data* and *Comparison of simulation and monitoring on a coenological state-plane*.

Seasonal dynamic patterns represented by field data

For the survey of empirical seasonal dynamic patterns, the data series of the years 1996 and 2002 are the most suitable, because sampling in these periods took place weekly or fortnightly. Observation data from the rest of the years (sampling monthly or more rarely) are suitable for testing hypotheses. In the series of data from 1996 (the year with the greatest sampling frequency from the aspect of Heteroptera) the proportion of the eight indicator organisms (larvae and adults of the four constant-dominant species) in the whole Heteroptera community, and the information content applying to the coenological change of state of the Heteroptera community can be well demonstrated. A great advantage of the 2002 series of observations are that they provide a possibility to compare the coenological behaviour (similarity pattern of the phases of the series of change of state) of the 8 dominant indicator organisms and the absolute dominant (non-Heteroptera) species of the aquatic macroinvertebrate community.

Empirical patterns of the year 1996

For the characterization of the seasonal change of state, firstly the formation of the summarised indicators is worth to be surveyed. All of the indicators have been counted for the whole aquatic and semiaquatic Heteroptera communities and separately to the eight indicator organisms as well. We signed the latter on the figures with a comma in the upper index. In *Figs. 2–5*, values of total number of collected individuals, total number of kinds of living organisms (species + their development stages), $\log_{10}$ sum of total individual numbers, and Shannon diversity can be seen. For the course of individual numbers, it can be stated that approximately until the 250th day of the year – with fluctuations – they grow nearly linear in their trend, and after this date, steeply and near linearly again they drop. It can be also observed, that the two curves of individual numbers diverge from each other in the greatest extent in the growing phase; but in the phase of decline they draw very near. This means that the non-dominant species emerge in great numbers at a time, when the dominant species are still in intensive growth. (Presumably they do not exploit at this time the available resources, so there's a weak competition pressure). The number of kinds of organisms, which is displayed in *Fig. 3*, strengthens this theory,

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amended with the fact that the number of kinds of organisms reach the peak around the 140th day and after this date – with small fluctuations – they remain on peak until the previous date of the peak of individual numbers. Running of the curves of *Fig. 4*, which displays the sum of logarithms shows a transition between the formerly discussed two figures – this is in line with expectations because we apply logarithm in order to recognize changes in order of magnitude. In this figure, the gradual diversion in the growing phase, and the approach in the declining stage of the two curves can be observed at best, which points to the essence of the pattern in a more sophisticated way. *Fig. 5*, which displays the behaviour of the diversity indices, makes it clear that after reaching the peak of number of kinds, the reduction in diversity is caused by the decline of the evenness of the frequency distribution. So, after the 150th day, the dominance of the dominant species grows firmly. And this also means that primarily the dominant species participate in ill-proportioned extent from the fast growth of individual numbers. Decline in individual numbers occurs by the conservation of these proportions, so during the year the development stages of the 4 constant-dominant species gradually crowd out the others.

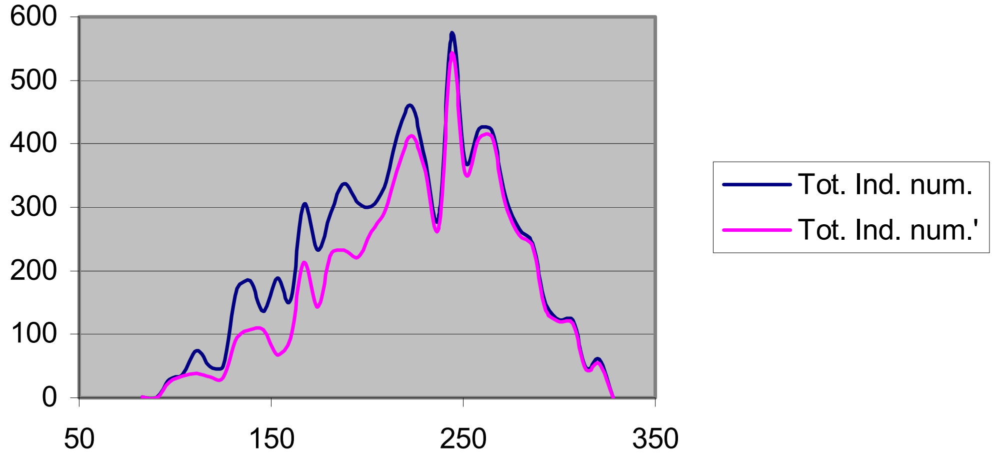

Figure 2. *Total individual number of samples in 1996*

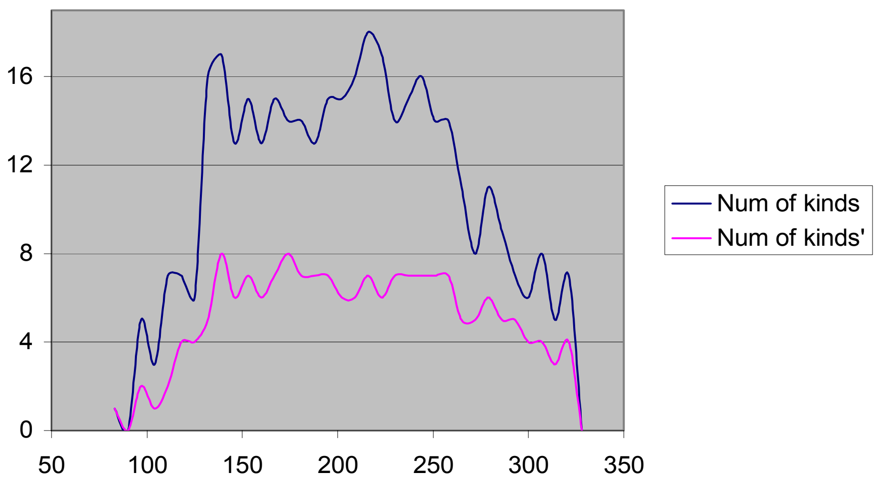

Figure 3. *Number of kind of living organisms (development stages of Heteroptera species) found in samples in 1996*

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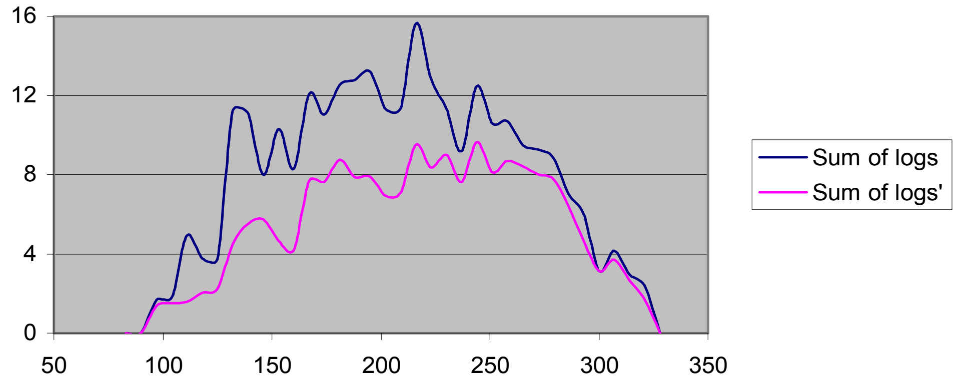


Figure 4. *Sum of individual number logarithms of number of kind of living organisms found in samples in 1996*

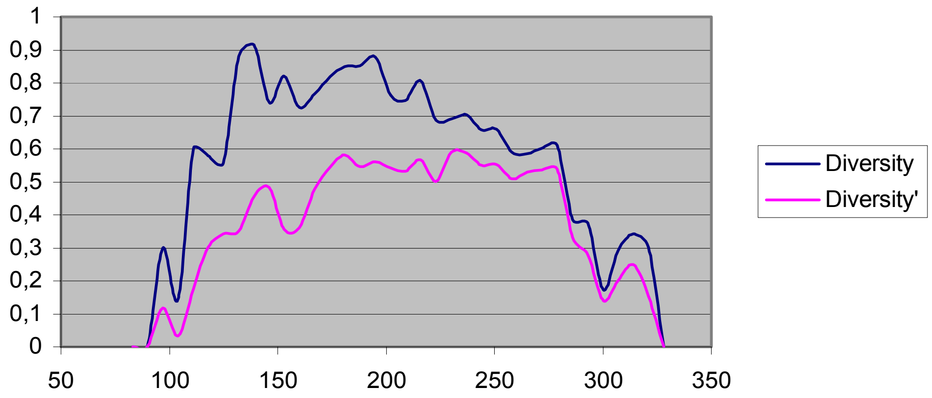


Figure 5. *Values of Shannon-diversity in 1996*

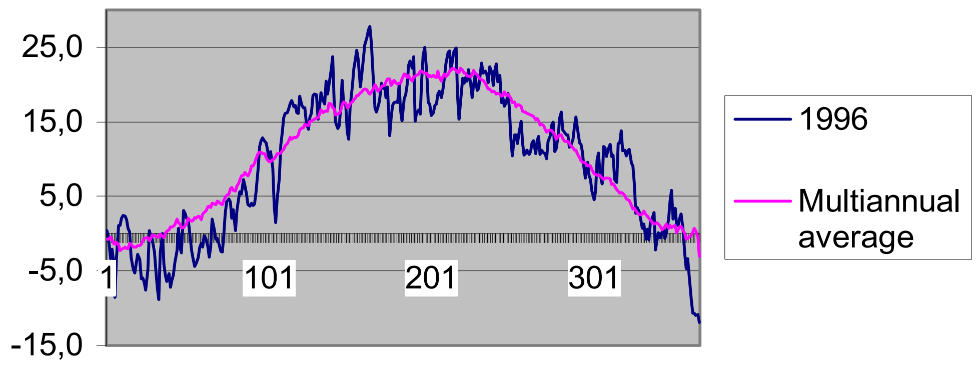


Figure 6. *Daily mean temperatures in 1996 and the multiannual average of daily temperatures*

In *Fig. 6* the daily mean temperatures are displayed. It can not be disregarded, that the shape of this curve is quite parallel to the afore-mentioned curves. This supports the theory that temperature has a decisive role in the forming of seasonal patterns.

Conjugated indicators, which unify variables by different methods help to give a picture of the process of change of state, but the conjugations hide the similarity patterns of

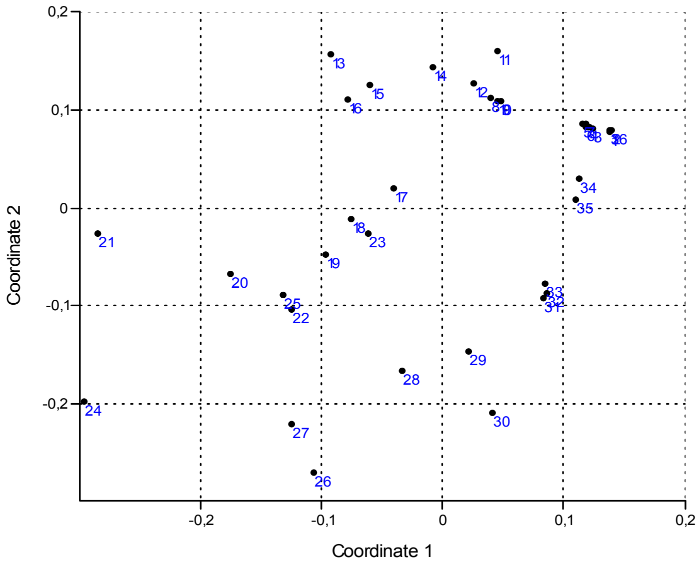


Figure 7. *Coenological changes of state of the Heteroptera community in 1996. NMDS ordination with euclidean distance. Serial numbers on the chart denote sampling dates from the 12th week of 1996, taken daily, by Saturdays.*

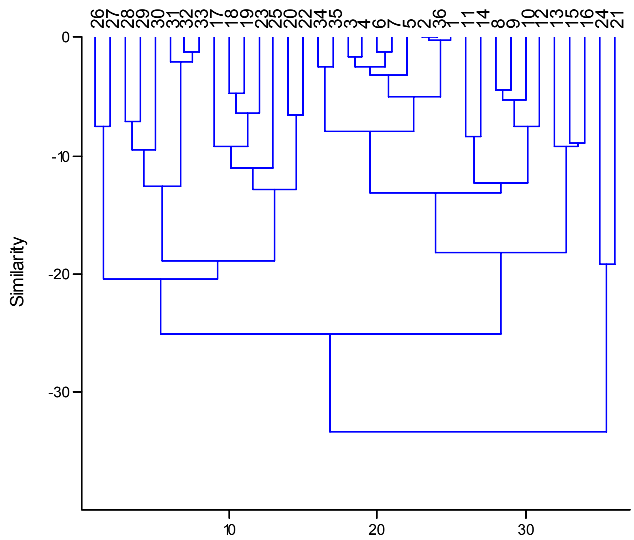


Figure 8. *Classification of the samples from the 1996 year with UPGMA method (group averages). (Serial numbers are identical to the numbers in Fig. 7)*

coenological states. To the analysis of temporal similarity patterns of coenological states, the application of multidimensional dimension-reducing and classifying methods lead us nearer. Pattern analyses have been carried out for the whole of the Heteroptera community and separately for the selected indicator organisms in order to test the predictive power of the patterns of indicator organisms. The similarity patterns have been explored with NMDS (*Figs. 7* and *9*), and for the testing of groups, cluster analyses have been carried out (*Figs. 8* and *10*) by applying the same distance function. Reliability of ordinations have been justified even with Shepard-diagram (*Figs. 11* and *12*).

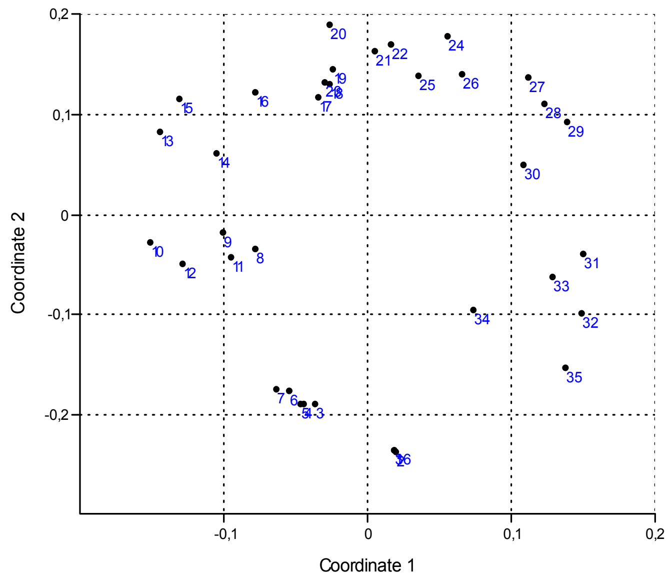


Figure 9. *Coenological changes of state and similarity pattern of dates of the indicator organisms from the 1996 year. NMDS ordination, euclidean distance. (Serial numbers are identical to the numbers in Fig. 7)*

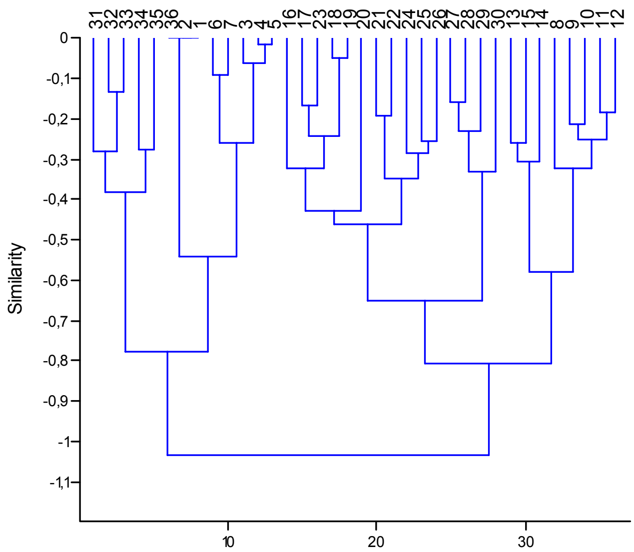
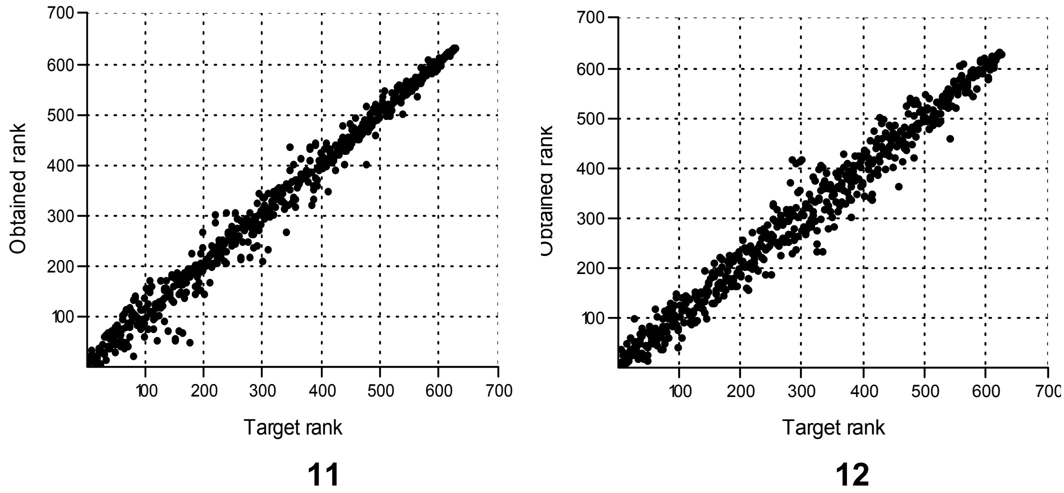


Figure 10. *Classification of samples by indicator organisms from the 1996 year, with UPGMA method, euclidean distance. (Serial numbers are identical to the numbers in Fig. 7)*

It can be observed in both of the ordination figures, that during the vegetation period (and practically during a year), coenological states describe a cycle of change of state which explains seasonality. *Fig. 9*, which displays exclusively the constant-dominant indicator organisms, shows a more regular trajectory than *Fig. 7*, which displays all species of Heteroptera.

The reason for this is that forms with little frequency (closer to the sampling threshold) are stronger influenced by random admission (noise of information) into



Figures 11, 12. *Shepard-diagram to* 11: *Fig 7 and* 12: *Fig. 9.*

samples. However, this can not hide the basic pattern-generating processes. Another important and well-studiable pattern characteristic is (apart from seasonal cycle), that points representing the samples are not evenly positioned along the line of the imaginary trajectory, but they form small groups. It can be well observed by comparing ordinations and classifications pairwise, that these groups are not artificial analysis products, but units of the real pattern. Using the traditional concepts of coenology [15, 16] these groups can also be called as aspects. Within the aspects the changes are slow, but at the time of changing of aspects, changes become fast.

Hypothesis system and model for the interpretation of the patterns gained

During the evaluation of the series of field data, temporal (seasonal scale) pattern of the coenological changes of state of the aquatic and semiaquatic Heteroptera community has been drawn. Results of the analysis indicated that temporal changes of weather parameters may have a decisive role in the determination of the patterns. Moreover, a very important result is that by selecting the larvae and adults of the four constant-dominant species of the Heteroptera community, we get eight indicator organisms, which seasonal behaviour is of predictive power for the seasonal changes of state patterns of the whole macroinvertebrate community. Thus, the similarity pattern shown by the seasonal changes in the state of those Heteroptera species providing the indicator organisms resembles to the pattern shown by dominant (non-Heteroptera) organisms. Presumably the strong ecological pattern-generating role of weather stands behind this phenomenon. The descriptive analysis of the patterns can only foreshadow the cause-effect relations, but it is incapable of exploring them. The complete exploration of the complicated interaction system which exert its effect inside the community would require the work of many researches during many decades. Between the frames of this current work however, it seems to be a realistic task to summarise – obviously by a strongly simplified hypothesis-system – the presumptions to the causes of the seasonal behaviour of indicator organisms. During the formulation of the hypothesis-system, the task is to grab and to stress the most important phenomena, which can be achieved by disregarding or – in a strongly simplified manner – conjugating the greatest part of interactions, which are otherwise interesting and important. To test the interpreting power of the hypothesis system, a mathematical model of the hypothesis system has to be formed in such a way, that it should be directly comparable with the observations.

The objective of the model is to examine, whether the seasonal change of daily mean temperatures in itself could cause seasonal coenological change of state pattern, which resembles to the results of series of field observations.

In our previous works, we worked out general simulation model-systems for the modelling of the seasonal-dynamic processes of communities (see subsection of literature overview). The best summarising description of the model-system applied here can be found in the paper [96]. For the modelling of the Heteroptera community on Szilas stream, we wanted to formulate such a hypothesis-system, which provides the possibility to adopt our general model-system to the current situation (see methodological sub-section: Methods of simulation modelling) and simultaneously defines the validity limits of the model.

According to the above-mentioned objectives, the following hypothesis- and condition-system were formulated:

1. The biomass-dynamic of the populations of different species is determined basicly by the change of daily mean temperature.
2. Strength of population interactions (direct consuming relations, source competition, apparent competitions, other direct interactions) and their resultant effect influencing a given population are also a function of daily mean temperature.
3. Feeding network of the community is decisively based on detritivor organisms, thus the main system-ecological processes are donor-controlled, but the detritus stocks are always abundant.
4. Structure of the dominant species of the observed habitat can change only in proportions but these dominant species locally do not extinguish and later arriving (migrant, invasive) species do not become dominant (or if they do, this does not influence the examined seasonal-dynamic processes).
5. Occurrence dates of phenologic (and individual development) phases are exclusively determined by an effective heat sum which is specific for the given phase (heat sum unit), and the physiologic condition of the given individual.
6. Individuals with different physiologic state in the population constitute a small number of discrete sub-populations.
7. Differences in the physiological state of the individuals in the populations determine only the starting values of the additive heat sum units (condition, stored quantity of nutrients), but there is no difference in the speed of accumulation.
8. Actual number of the indicator organisms is only determined by the local phenologic changes, and the possible immigration or emigration from the imminent proximity (migration). In respect of phenologic stages, nearly identical states are present in the neighbourhood of the examination spot.
9. Migration can only level off those differences, which are actually existing between the temperature-dependent potential and the locally and historically developed number of individuals. (Thus, the arriving immigrants beyond the subsistence level move away or die instantly, but the headcount up to the possibilities can be filled even by migration).
10. Weather phenomena occurring in winter – before the 80th and after the 340th day of the year – do not influence the processes in the vegetation period because of the diapause characteristics of the species. (Individual development stages do not take place even if the weather should be otherwise favourable).

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11. All the observed species overwinter exclusively in adult form.
12. Apart from the above-mentioned factors other (disregarded) factors do not effect the community.

The hypothesis-system above can be formulated mathematically in the following model:

$$X_{i,t} = \begin{cases} \log_{10} A_{i,t}, & \text{if } A_{i,t} \geq 1 \\ 0 & \text{otherwise} \end{cases};$$

$$i = 1, 2, \ldots, 8;$$

$$t = 1, 2, \ldots;$$

$$A_{it} = \sum_{t-5}^{t+5} \frac{B_{it} \cdot C_{it}}{11};$$

$$B_{lt} = R_{lT} \cdot B_{lt-1};$$

$$l = 1, 2, 3, 4;$$

where

$X_{i,t}$: order of magnitude of the predicted individual number of the i^{th} kind of organisms on the t^{th} day (this is desired to be gained with the simulation);

$A_{i,t}$: number of individuals (i^{th} kind of organism on the t^{th} day);

$R_{l,T}$: a constant ordered to temperature intervals, which is a parameter of the model;

i: index of the kind of organism (given development stage of a given species);

l: index of the species;

t: serial number of a day in a year;

T: daily mean temperature;

$B_{i,t}$: actual value of the biomass of the population;

$C_{i,t}$: proportion of the individual development stage in question in the population of the species.

$$C_{i,t} = \frac{\sum_n D_{itn}}{\sum_n D_{itn} + \sum_n D_{jtn}},$$

$$D_{itn} = a_n \cdot E_i(F_{nt}),$$

where

n: index of sub-populations;

a_n: a constant characterizing the magnitude of the n^{th} sub-population,

E_i: switch function, which can take only the values of 1 or 0; 1 if it is in the range of the F_{nt} effective heat sum unit model, and 0 if it is outside the range;

D_{itn}: mass indicator of the given individual development stage of the given species (i^{th} kind of organism);

D_{jtn}: mass indicator of the other development stages of the given species.

$$F_{nt} = d_n + \sum_{k=t_0}^{t} T_k,$$

where

d_n: condition of the n^{th} subpopulation (starting value of the heat sum measurement);
t_0 **:** starting day in the spring (here, the 80th day of the year);
T_k: value of the °C degrees above the species-specific base temperature on the k^{th} day.

With the model above, the change in the individual number of species and their development stages can be easily calculated if we write the equations into one row of the MS Excel (into cells referring to each other) and copy them below each other so many times, as many days we want the simulation to be calculated. We wrote the parameters into a separated part of the table and referred to them in the model with cell-references. For the optimalization of the parameters the MS Solver program were used, where conditions (validity limits) can also be defined.

Comparison of simulation and monitoring on a coenological state-plane

With the method described in the methodological subsection, a reference table from the daily coenological data of years with very different weather was established, from which, by applying NMDS, coordinates were ordered to the indicator organisms. The gained coordinates can be seen in *Table 4*. Later only these were used for the coenological state-planes. On the state-plane, coordinates of the samples were defined with the sum of the appropriate coordinates multiplied by the logarithms of individual numbers of indicator organisms.

Table 4. *State-plane coordinates of the indicator organisms (indicator coordinates)*

Indicator organism	X coordinate	Y coordinate
Nepa cinerea adult	-0.258	0.105
Nepa cinerea larva	0.102	0.222
Notonecta glauca adult	0.116	-0.492
Notonecta glauca larva	-0.265	0.150
Gerris lacustris adult	0.384	-0.150
Gerris lacustris larva	0.417	-0.108
Hydrometra stagnorum adult	-0.241	0.120
Hydrometra stagnorum larva	-0.253	0.154

Since the indicator coordinates are located around the origo (in all plane quarters) their weighted sum can be interpreted as linear combination of vectors pointing from the origo towards the indicator coordinates. Values of coenological coordinates characterising the sample are also influenced by the proportion and quantity of indicator organisms.

Coordinates of the coenological state-plane can be computed even to the empirical field monitoring data, and to the data which were simulated by the model from the daily mean temperatures of the same year. Thus the model and the empirical experience can be well compared.

Case-study of the year 1996

In *Fig. 13*, the empirical and simulation data from 1996 are displayed on coenologic state-plane. It can be well observed on the coenologic state-plane that the seasonal cycle appears on an elongated, oblong trajectory, with spring and autumn points positioned near to the origo, and midsummer points located on the furthest position from the origo in the right bottom plane quarter. The growing phase in the spring and the declining stage in the autumn runs differently, which meets expectations gained from former experiences (see subsection: *Seasonal dynamic patterns represented by field data*). Points from field samples are positioned around the model-trajectory, the two series of data fits well to each other. So, the model approaches reality quite well. In *Figs. 14* and *15*, simulated and empirical individual number logarithms of indicator organisms are even together and separatedly displayed. Comparing the seasonal patterns by species of the two figures, good accordance can be observed between the two series of data. Though a remarked difference is that the course of the curves of the model are simpler, and less hectic than the empirical series of data. It is quite conspicuous because the empirical series of data contain only weekly observations, while the model gives daily simulations. Thus, it can be stated that although the model shows the behaviour of the system, some of the disregarded interactions should be considered during the later development of the model.

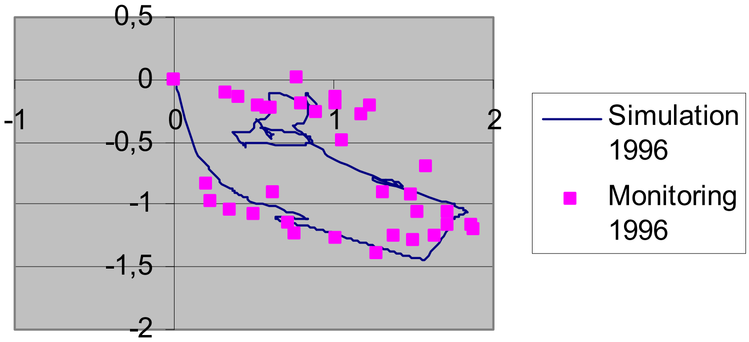

Figure 13. *Comparison of field and simulated data on coenological state-plane (1996).*

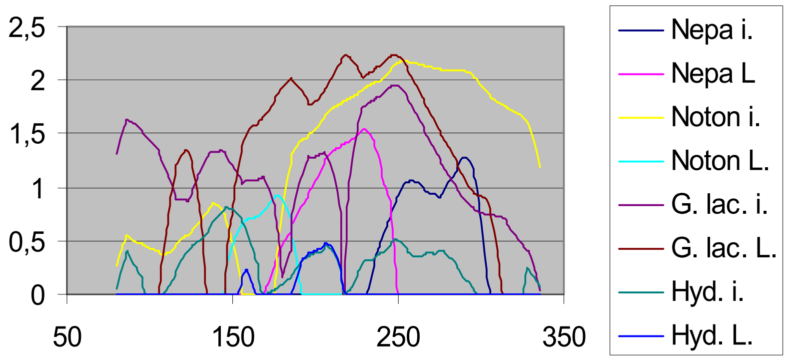

Figure 14. *Simulated data of the individual number logarithms of indicator organisms (1996).*

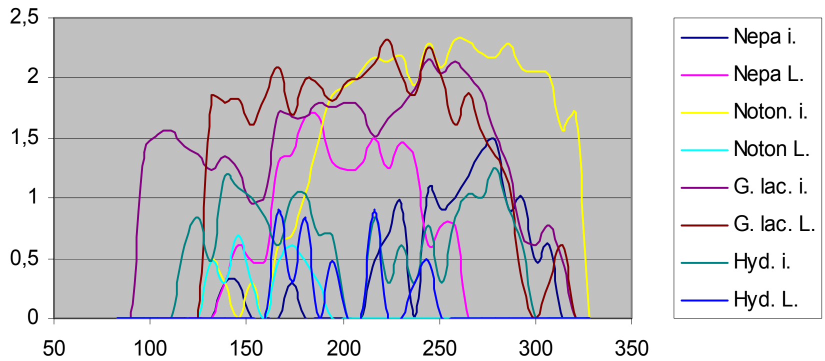

Figure 15.: *Empirical data of the individual number logarithms of indicator organisms (1996).*

In *Fig. 16*, individual number logarithms from simulation and monitoring are displayed. Based on the figure, it can be stated that the model behaves realistically even in this respect, but processes of the field series of data run down faster and the model slightly underestimates the individual numbers in the growing phase.

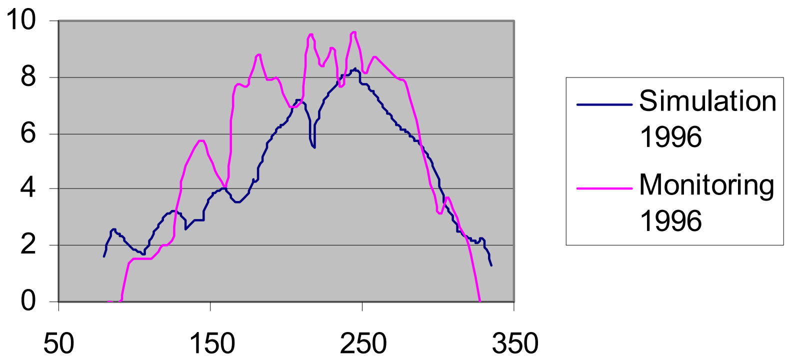

Figure 16. *Sum of individual number logarithms of indicator species in case of 1996 empirical and simulated data.*

Climate change scenarios and the seasonal dynamic of the Heteroptera community

An advantage of the simulation models is that they are capable of giving predictions to such kind of situations, which empirical examination is not possible. Before further developing the model, we considered to be practical to thoroughly examine how the model works under such weather circumstances which are basicly different from the kinds experienced here. Besides, these simulation experiments are also suitable for exploring – by taking into consideration the known errors and validity limits of the model – the most possible scenarios of climate change. Obviously, (on our present level of knowledge) we do not have the possibility to forecast the future – even in the respect of the Heteroptera community on Szilas stream. However, it can be considered a realistic objective to examine the predictions of our model to realistic, hypothetical, or such kind of data series, which were generated by other models. This research objective fits into the goals of the VAHAVA project of the Hungarian Academy of Sciences, and also to the currently accepted main trend of the international climate change research [18, 32, 56, 100, 185].

To explore the possible effects of a future (such of unknown rundown) climate change, not real, but of realistic kind alternative scenarios, so-called climate-scenarios are needed. To clearly define the concept of climate change, we consider the following definition acceptable: „An inventory of future climates, which were made by using scientific principles, each of them is consistent – in itself – but none of them possesses a definite probability in accordance to which it shall take place "hence climatic scenario is only one of the possible climates and by no means prediction." [17, 55].

Recognizing the ecological problems of climatic change paved the way to the foundation of IPCC (Intergovernmental Panel on Climate Change) organisation (established by WMO – World Meteorological Organization – and UNEP), which aim is to give extensive and objective information about the scientific grounds and expected changes of a possible climatic change. During our work, we considered the principles determined by IPCC and used some of the internationally best accepted scenarios.

Climatic change scenarios which can be considered serious, are results of simulation models. For scenario generation, the so-called GCM-s, (General Circulation Model or Global Climate Model) are used.

In the present paper, the GFDL2535 and GFDL5564 scenarios of the American Geophysical Fluid Dynamics Laboratory – which take the atmospheric CO_2 rise into account, the BASE scenario – run with the current conditions, and the three very different scenarios of United Kingdom Meteorological Office (UKMO), namely UKHI (high resolution equilibrium climate change experiment), UKLO (low resolution equilibrium climate change experiment) and UKTR (high resolution transient climate change experiment) were used. The first two from the UKMOs three scenarios are so-called equilibrium models, which presumes the doubling of atmospheric CO_2 and the models run until they get an equilibrium state of energy and stable surface temperature. UKTR is a so-called transient model, which examines the gradually changing climate by gradually growing atmospheric CO_2 content [18, 81].

Rundown results of GCMs have to be scaled down to the given observation region with the use of empirical-statistical relations [32]. In this work, uniformly the 15th running year of the 30-year-long models (applying to the period around 2050) were chosen. Its scaling down to the Hungarian region was made originally to the CLIVARA project (Climate Change, Climatic Variability and Agriculture in Europe). The necessary database and literature were provided by professor Zsolt Harnos, leader of the Hungarian project.

Simulation experiment with the homogeneous manipulation of multiannual average of daily temperatures

Before the examination of climatic change scenarios, we wanted to test the climatic sensitivity of the Heteroptera community model with simpler, easier to interpret, manipulated data series. In order to achieve this, the data series of multiannual average of daily temperatures were calculated, and by adding and subtracting 2 °C, three different data series with identical shape but different values were gained.

In *Figs. 17* and *18*, the results simulated by the three data series are displayed. In *Fig. 17*, change of state trajectories can also be seen on the coenological state-plane. The effect is powerful, and has a well-recognizable pattern. By the reduction of temperature, the trajectory elongates, by growing temperatures it widens compared to the main axis of the original trajectory.

In *Fig. 18*, the sum of individual number logarithms of the indicator organisms is displayed. Rising temperature causes growing density, while the decline renders the growing process steeper, so at the beginning of the stage is under the original values, and in the vicinity of the peak, it exceeds them. The three data series of temperature not only changed the magnitude of the values, but also altered the rundown of the curves.

Taking into account that according to former tests, the simulations of the model underestimated the individual numbers during a warm period, it is quite spectacular, to what extent individual numbers grow with the rise of temperature. If we consider the former experiences, we should think that a rise of temperature in reality would have an even greater impact than predicted by the model.

Simulation experiment with scenarios of global atmospheric models scaled down to Hungarian regions

Comparing the alternative climatic scenarios of the period around 2050 to the realistic meteorologic data series from the 1990's, the simulations conducted by the model show a marked difference even in respect of trajectories which can be observed on the coenological state-planes (*Figs. 19* and *20*), and even in respect of sum of individual number logarithms (Figure 21. and 22.). Simulated trajectories of scenario-

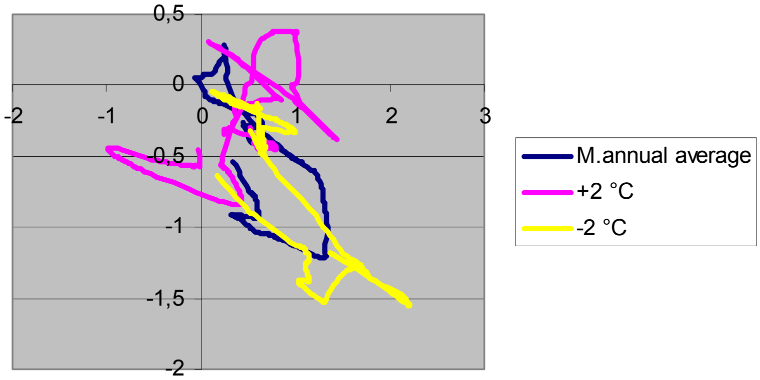

Figure 17. *Simulated change of state trajectories gained by homogeneous modification of multiannual daily average temperatures on a coenological state-plane.*

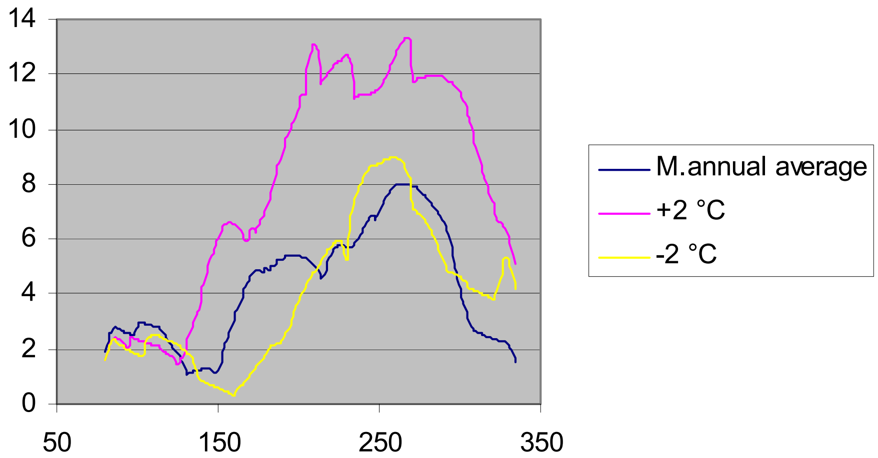

Figure 18. *Sum of individual number logarithms of simulated data series gained by homogeneous modification of multiannual daily average temperatures on a coenological state-plane.*

simulated data series show a much broader angle than the data from the '90s. Broader angles refer to a greater change in individual numbers.

By observing *Figs. 21* and *22*, it can be stated that scenarios predict the growth of individual numbers and earlier position of peaks compared to simulations based on realistic data. Prediction of the BASE scenario stands nearest to the curves simulated by data from the 1990's. BASE scenario gives a prediction by presuming unchanged level of atmospheric CO_2 (originally designed as control).

Simulation experiments by climatic scenarios pointed that the model designed for describing the seasonal-dynamic behaviour of Heteroptera indicator organisms is capable of giving appealing, well-interpretable realistic predictions by sensitively reacting for weather situations, which basicly differ from the ones which had been experienced during the validation of the model.

Discussion

In the community examined in this work – considering either only the Heteroptera, or the entire invertebrate species community – strong dominance of small number of

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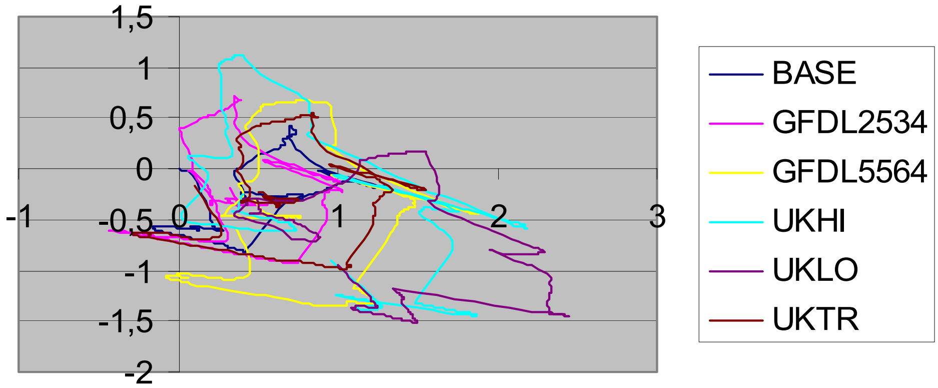

Figure 19. *Expected rundown of trajectories of coenological change of states around 2050 by simulation based on different climatic change scenarios.*

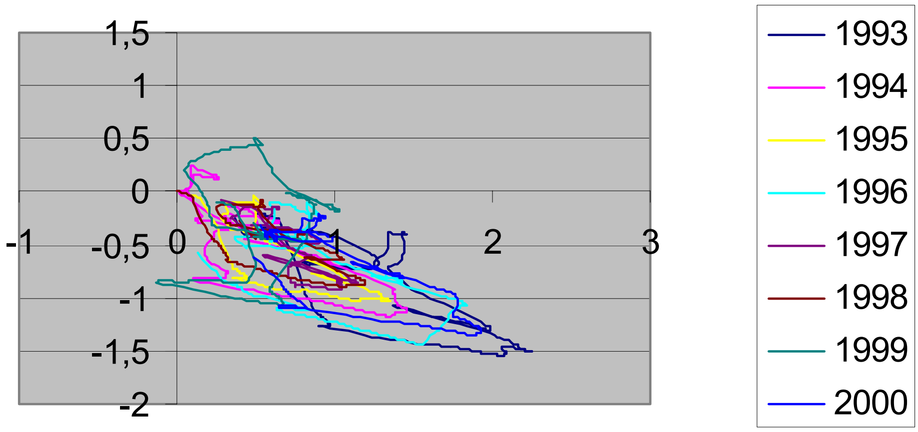

Figure 20. *Rundown of trajectories of coenological change of states around 2050 by simulation based on real data series from the 1990s.*

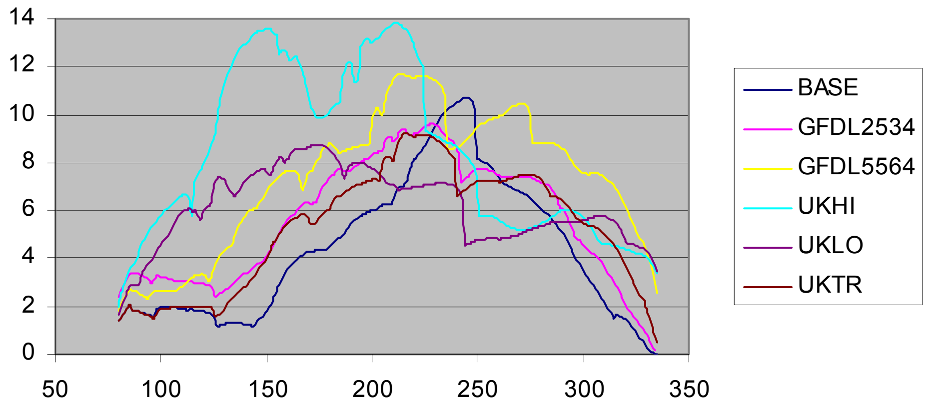

Figure 21. *Expected rundown of sum of individual number logarithms around 2050 by simulation based on different climatic change scenarios.*

dominant species can be observed beside the perceivable presence of relatively numerous species. In other aquatic habitats which we have examined, either a much lower number of species (and thus, low diversity) or much higher evenness beside approximately the same number of species (and thus, higher level of diversity) could be observed. The

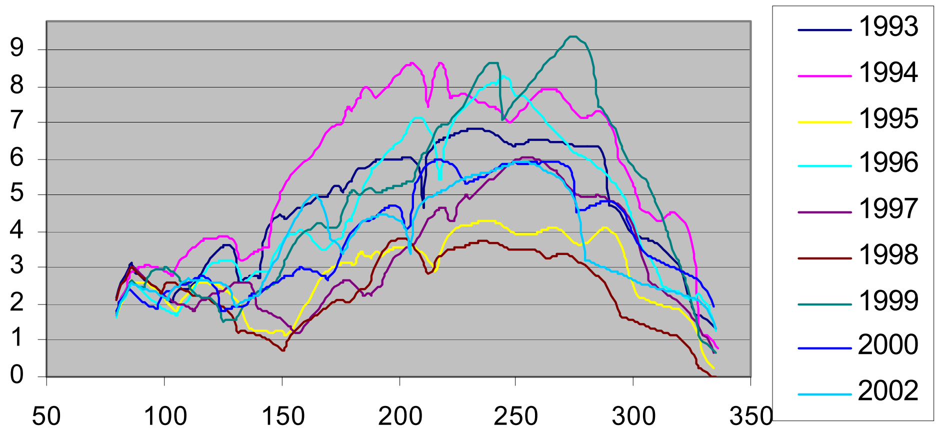

Figure 22. *Expected rundown of sum of individual number logarithms around 2050 by simulation based on real data series from the 1990s.*

reason for this presumably lies in the fact, that although this section of the Szilas stream can be considered a small current regarding its size, however it has a much greater hydrologic stability compared to other streams, which is mostly a feature of waters of greater size. A slight setback of the dominance of dominant species occurs approximately between the 150th and 200th day, when the species becoming more and more dominant during the year can not entirely exploit the disposable resources for historical reasons (delay in their prolification compared to ecological possibilities). In this period, level of diversity enhances, which is demostrated in the greater measurable number of species, and the lesser distributional unevenness. Later these species become gradually crowded out and even their lifecycle would not be always completed. For some species, the great number of migrating individuals is characteristic, this explains the ill-proportionedly great number of adults compared to the number of larvae.

The cyclic feature which is characteristic to the seasonal coenological change of state pattern of species communities, is probably determined primarily by the circaannual rundown of temperature. It is very improbable, that the rhythm in temperature and community structure would commence by the identical influence of a third, common factor. Thus, a direct cause and effect relation is presumed, though we could not prove it. A characteristic feature of the change of state pattern is – apart from its cyclic attribute – the appearance of aspects (similarity groups). The real reasons and ecological significance of this phenomenon is not meritably explored until now.

An important statement of this work is that based on the behaviour of a relatively small number of selected species the behaviour of the entire community can be assuringly predicted. Practically, this means that by monitoring a small number of indicator organisms, the change of state of the entire community may be surveyed. From our point of view, the high level of indicability of the seasonal patterns is an independent pattern-generating factor (presumably the temperature) which exerts the same effect on every population, and not by the cause and effect relations between the variables (e.g. population interactions).

Our hypothesis applying to the role of temperature is strengthened by the fact, that very resembling results can be simulated with a model which disregards all other outer abiotic factors and population interactions, despite it applies a very simple mathematical apparatus.

Applicability of the stable state-planes based on indirect ordination is supported by the strong rules discussed above and the variable conditions which can be simulated by the model. The stable state-plane system is capable of effective dimension-reduction only in case when a broad spectrum of possible states is involved into the reference database which serves as a base for coordinates. For this, either many years of monitoring, or appropriate and realistic extension of the results of monitoring is needed. Appropriate extension of monitoring data can only be achieved by the application of suitable models.

This simulation model shows numerous imperfections, thus its further testing and developing is needed, which can be achieved during further field examinations and manipulative experiments. In its current form, the examined phenomenon is formally exact, but less accurate in predictions.

Combination of the simulation model of the Heteroptera community with climatic change models, provides a possibility to conduct a series of simulation experiments which applies to the near future (to the period around 2050). In this experiment, a comparison of the known weather data from the 1990's, and the alternative scenarios for the future take place.

Results of the simulation experiments predict a growth in the individual numbers of the dominant species of the Heteroptera community, and earlier occurrence of phenological phenomena for the case of Hungarian aquatic communities resembling to those examined on Szilas stream.

Available alternative scenarios of global atmospheric models – which are best accepted by the international scientific public opinion – describe the expected climatic change on our planet in very different ways. Thus, their predictions to the future are considered rather uncertain. However, in case we intend to analyse the effect of a possible climatic change to different communities and ecosystems, we do not have any other possibility as the application and development of simulation models. Considering the vast social significance and its characteristic to fundamentally determine our existence we think that even the most uncertain, however realistic prediction based on uncertain probability scenarios worths much more, than the entire lack of information, since this provides a possibility to create newer hypotheses and more careful consideration of the possibilities to counteract.

Theses

Research and development results of our work and the statements and methodical substances from the case studies can be summarized as listed below:

1. Between 1991 and 2002, on the investigated section of Szilas stream among the observed 57 Heteroptera species 42 are known to conduct a lifecycle which is to some degree tied to water, and among these only six species had dominance exceeding 1%. From this six species, only four can be considered both in larva and adult stages as strictly constant elements. Thus, the Heteroptera community of Szilas stream can be characterised with great dominance of the constant-dominant species in absolute terms.
2. The *Gerris lacustris* and *Nepa cinerea* populations on the observed area can be considered as autochtones. Specimens of the *Notonecta glauca* and *Hydrometra stagnorum* populations can be considered as of partially autochtone and partially allochtone origin.
3. It can be stated that the greater proportional appearance of non constant-dominant species can be expected around the period of 150–200th day, which is

well before the appearance of the peak of the individual number of dominant species (generally around the 250th day).

4. For the seasonal dynamics of the whole Heteroptera community, the annual cyclic feature is predominant which is manifested in the sampling dates similarity pattern. Typical pattern element is furthermore the grouping of the temporally close dates into aspects. Inside the aspects relatively slower, between the aspects faster coenological changes can be observed.
5. It can be stated, that the seasonal coenological similarity patterns displayed by the adults and larvae of the four constant-dominant species selected as indicator organisms greatly resemble to the dominant (non-Heteroptera) macroinvertebrate similarity patterns typical to Szilas stream. The two variable groups can be brought into strong canonical correlation with each other. Thus, the collective behaviour of the indicator organisms has significant predictive power and information content which can be applied for the whole community.
6. As a substance concerning the methodology of field monitoring it can be stated, that the seasonal-coenological sampling frequency in case of macroinvertebrates should take place at least fortnightly (or weekly) from the aspect of recognizability of changing of patterns. If sampling takes place less frequently, important pattern elements will remain hidden, or due to the rapid changes between aspects, even a false picture can be drawn if rare sampling is conducted in a period when intensive changes occur.
7. Based on data, total individual number of the community and the sliding average of average daily temperatures correlate with each other. Temporal course of indicators of temperature and community structure changes resemble to each other. Maximum of sum of total individual number of the community and logarithm of individual numbers generally takes place after the maximum of daily average temperatures, but the maximum of diversity indicators commences earlier.
8. Based on data series of daily average temperatures as input parameters, such a discrete-deterministic model can be drawn, which is able to generate textural (community structure) data series which resemble to the empirical data series. Thus, based on the change in temperature as a pattern-generating factor, empirical patterns become comprehensible. The constructed model can be fitted to the real data series – provided its parameters are in biologically acceptable ranges. Among circumstances which are quite different from the experienced the fitted model works stable and consequent and thus fulfils professional requirements.
9. The stable coenological state-plane which was constructed based on the principle of indirect ordination is suitable for unified handling of data series of monitoring and simulation, and also fits for their comparison. On the state-plane, such deviations of empirical and model-generated data can be seen, which could remain hidden based on the behaviour of conjugated indicators.
10. Significant differences can be observed in the course of trajectories and in the conjugated indicators by running the simulation model of Heteroptera communities first with the data series of climate change (UKMO and GFDL) scenarios applied for the period around 2050, scaled down for the area of Hungary – which is most accepted internationally – and second by the data series of the 90's – which were tested by monitoring.
11. According to the predictions of the models by around 2050, drastic change in individual numbers can be expected – provided that other conditions of the

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hypothesis-system remain unchanged. Furthermore, the maximum of individual numbers will commence 20–50 days earlier than at present. Most of the applied climatic change scenarios count with the doubling of the aerial carbon-dioxide level. In case the carbon-dioxide level remains the same (BASE scenario), then by applying the computations of the global circulation model (GCM) and our Heteroptera coenological model, the circumstances around 2050 will resemble to the presently experienced figures.

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GROUNDWATER CHEMISTRY OF SHALLOW AQUIFERS IN THE COASTAL ZONES OF COCHIN, INDIA

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Abstract. The coastal aquifers of Kerala, India experience severe degradation of water quality due to various anthropogenic activities. An attempt is made here to study the groundwater chemistry of aquifers, which lie along the coastal zone of central Kerala. Results in general indicated that the groundwaters in the shallow aquifers were found to be deteriorated. Based on Hill-Piper trilinear diagram it is confirmed that some of the dug wells were characterised by high amount of sodium and chloride (>200 mg/l) indicating the influence of saline water incursion. The presence of *E. coli* in all dug wells indicated potentially dangerous fecal contaminations, which require immediate attention. The study further raises points for the need of action for a sustainable utilization of precious resources.
Keywords. *coastal aquifer, groundwater, trilinear diagram, saline water incursion*

Introduction

Kerala, the southernmost state of India has unique hydrogeological characteristics with wide variation in the rainfall pattern (average 3107 mm). Both qualitatively and quantitatively, the coastal zones of Kerala in recent years witnessed serious groundwater problems [8, 9, 11, 24, 25]. Several studies invariably showed that water quality in the shallow aquifers situated in the coastal zone of Kerala is deteriorating alarmingly amidst plenty of water all around [1, 6, 7, 10, 12, 21, 33]. Owing to the high demand of groundwater to cater a large population in the coastal zones of Cochin, mitigation of the deterioration in the quality of groundwater in shallow coastal aquifers was initiated through groundwater recharge [30]. High population pressure, intense human activities, inappropriate resource use and absence of proper management practices leads into the deterioration. The coastal sedimentary formation serves as an excellent condition for aquifer and the average groundwater potential of this region is estimated to be more than 0.3 MCM/km [b]. In the shallow coastal aquifer, open wells are the dominant groundwater abstraction structures and the density of the open wells in the coastal area is high in the range of 400 wells/km^2 [30]. During rainy seasons, the sea becomes rough and encroaches towards land and during summer seasons the saline water finds its way through tidal channels and it admixes with shallow coast aquifers. So the qualities of water in the shallow and deeper zones become brackish [9, 20, 30].

Added up problems such as urbanisation, industrialization, unscientific landuse, lack of awareness of the people and saline intrusion all makes the quality of groundwater in Cochin coastal zone worsen. All these contribute to less recharge into the coastal aquifers thereby accentuating groundwater quality and the problem of salt water intrusion. The present investigation attempts to illustrate the scenario of groundwater quality and saline water intrusion during post monsoon (November 2003) in the coastal zones of Cochin.

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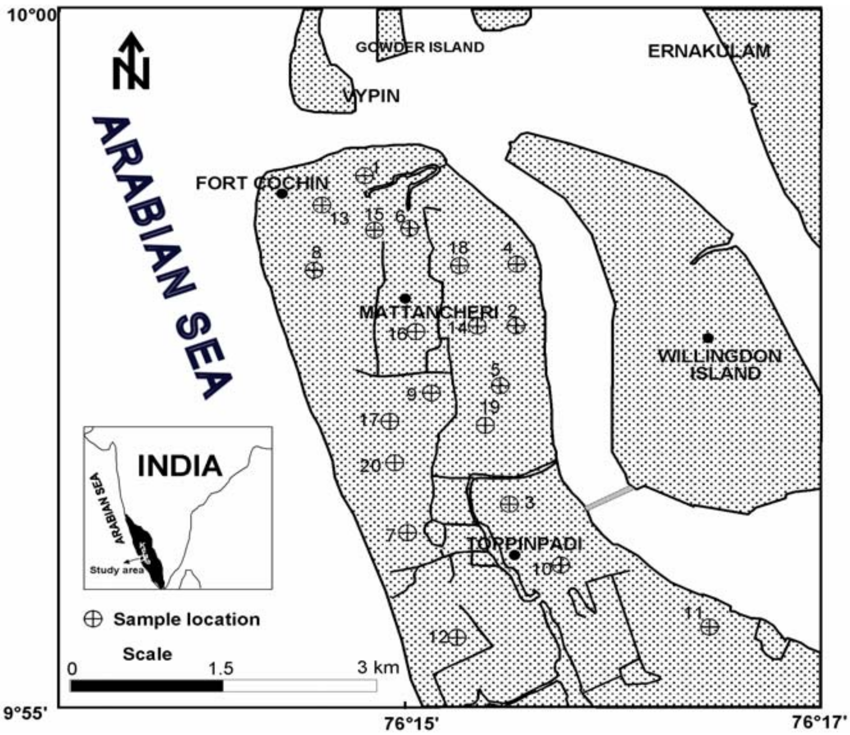

Figure 1. *Base map and sample locations of the study area*

Study area

The study area extends from north of Fort Cochin to the south of Thoppumpadi which lies between 9°55'–10°00'N and 76°13'–77°17'E (*Fig. 1*).

The area is bordered by Arabian Sea on west and a part of Cochin estuary in the eastern side. The area is characterised by a number of tidal channels, results into seawater encroachment, which deteriorate the water quality.

Exploratory borehole study conducted by Central Groundwater Board indicates the recent coastal alluvium followed by Tertiary sediments consists of two distinct formations. The upper most formation is Warkalais with thickness of nearly 80 m underlined by thick sequences of sediments called Vaikom beds. The Tertiary sedimentary formation of Kerala basin unconformably overlays Precambrians. In the present study most of the dug wells are tapping groundwater at depth ranging 2 to 8 m fall in recent coastal alluvium [29].

Data and methodology

Groundwater samples have been collected from 20 dug wells during post monsoon (November 2003) at stations as shown in *Fig. 1*. The pH was measured at the spot, whereas the concentration of major cations, anions and *E. coli* were analysed at the laboratory as per the standard analytical procedures [2, 14].

Sodium and potassium in groundwater samples were analysed using Flamephotometer (Systronics FPM digital model). Calcium and magnesium were estimated by EDTA titri metric method, whereas chloride was determined by argentometric titration using standard silver nitrate as reagent. Carbonate and bicarbonate concentrations of the groundwater were determined titrimetrically [2, 14]. Sulphate concentration was carried out following turbidity method using double beam UV-Visible spectrophotometer

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(Hitachi Model 2000) [2]. The microbiological quality of samples were analysed in terms of most probable number (MPN) of faecal coliforms using lactose broth and incubation at 44.5 °C. Tubes showing positive results after 24 to 48 hours of incubation were streaked on to Mac Conkey Agar and esoine methyl blue (EMB) agar and incubated at 37 °C for 24 to 48 hours. Typical *E. coli*-like colonies were isolated and confirmed biochemically as *E. coli* using IMViC test. The number was expressed as MPN index / 100 ml.

Results and discussion

Table 1 presents the results of groundwater analysis.

pH

The pH values of groundwater were varied from 7.01 to 8.2 indicating slightly alkaline nature. Groundwaters with pH value above 10 are exceptional and may reflect contamination by strong base such as NaOH and $Ca(OH)_2$ [22]. The range of desirable limit of pH of water prescribed for drinking purpose by ISI [27] and WHO [35] is 6.5–8.5 while that of EEC [23] is 6.5–9.0.

The analysed groundwater samples are within the limit prescribed by ISI [17], WHO [35] and EEC [23]. There is no much distinct variation of pH in the different wells selected for the present study, indicating that the groundwater is tapping from aquifers of a single formation. The slight alkaline nature of groundwater may be due to the presence of fine aquifer sediments mixed with clay and mud, which are unable to flush off the salts during the monsoon rain and hence retained longer on other seasons.

***Table 1.**: Chemical and E. coli analysis data of groundwater*

well no.	pH	Na^+ (mg/l)	K^+ (mg/l)	Ca^{2+} (mg/l)	Mg^{2+} (mg/l)	CO_3^{2-} and HCO_3^- (mg/l)	Cl^- (mg/l)	SO_4^{2-} (mg/l)	MPN index FC / 100 ml
1	8.08	380.7	23.5	57.9	20.2	200.2	470.4	1.8	150
2	7.61	145.7	90.6	102.1	12.0	317.8	145.2	2.1	290
3	8.01	480.4	110.0	190.4	22.7	334.1	749.4	5.6	95
4	7.67	457.1	75.2	117.7	10.3	321.9	640.3	2.4	95
5	7.10	120.0	18.2	72.9	22.5	352.4	36.1	1.8	460
6	7.14	110.9	19.8	74.1	26.5	310.1	63.5	2.2	290
7	8.20	546.0	72.2	122.0	11.2	276.9	757.4	3.8	93
8	7.26	90.7	30.2	52.8	5.0	179.7	96.7	2.2	460
9	7.30	70.6	38.4	48.8	4.8	187.0	49.8	1.1	460
10	7.36	445.9	45.9	70.2	11.0	160.1	612.0	3.4	120
11	7.53	110.6	38.3	50.8	15.3	203.6	128.2	2.5	240
12	7.60	339.0	95.1	87.0	22.8	187.4	541.6	3.5	150
13	7.57	111.5	65.2	54.4	3.6	185.7	126.0	1.9	210
14	7.20	65.0	23.7	82.6	3.5	238.5	23.7	0.9	240
15	7.67	85.3	42.5	97.1	2.5	234.0	70.6	2.1	210
16	7.54	64.5	15.2	61.4	1.2	167.4	37.5	0.8	460
17	7.50	34.0	8.9	24.6	2.3	77.0	23.1	0.6	290
18	7.07	20.1	10.2	25.6	1.7	62.5	26.0	1.2	460
19	7.50	48.5	27.6	83.2	10.1	202.2	55.4	1.3	240
20	7.01	11.7	5.2	34.0	8.9	74.1	25.2	0.9	460

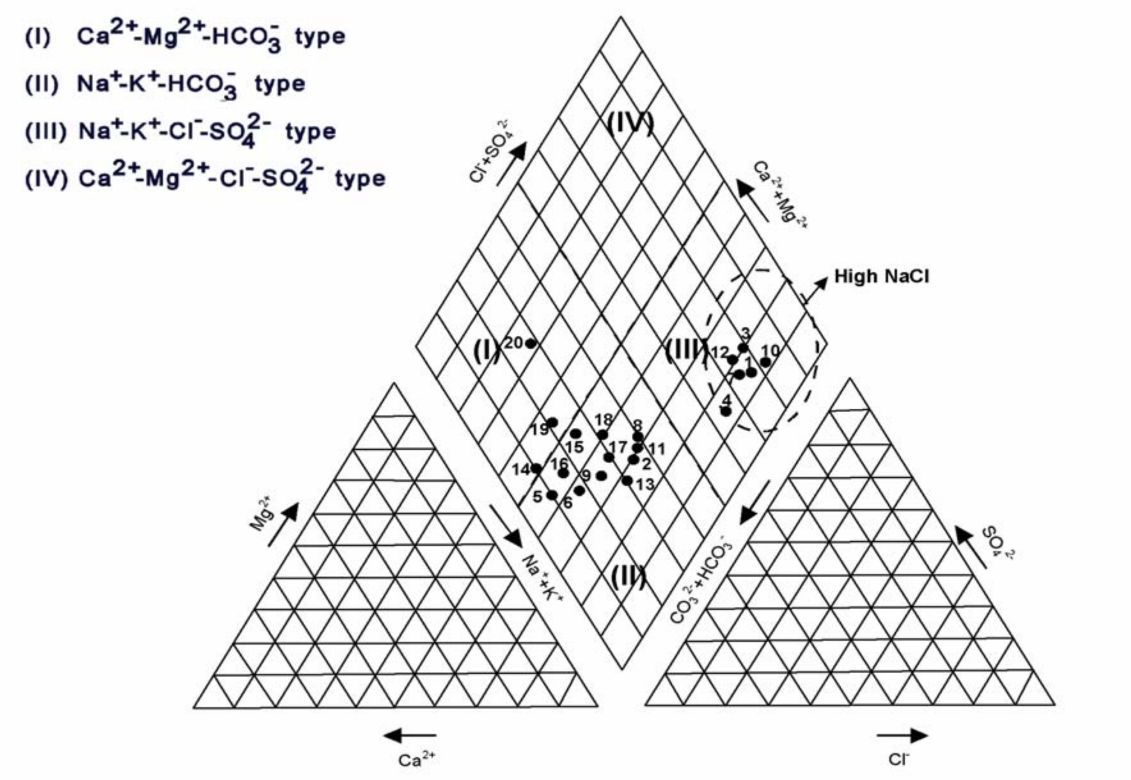

***Figure 2.**: Trilinear diagram of dugwell samples*

Major cations and anions

Major cations and anions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^-, CO_3^{2-}, SO_4^{2-} and Cl^- (*Table 1*) were plotted in hydrochemical trilinear diagram. In general high concentrations of chloride in groundwater is attributed to rainwater, seawater, natural brines, evaporate deposits and pollution [18, 19]. In dug wells (Nos. 1, 3, 4, 7, 10 and 12) high concentrations of chloride and sodium were measured. This high concentration can be due to the proximity of the wells to the tidal channel and the poor muddy sediments present in the aquifer system which further infers saline incursion. The high chloride content is generally taken as an index of impurity of groundwater. The clogging nature of sediments permit only intermittent flushing and hence the impurity (sodium and chloride) was sustained longer as compared to other wells. The dug wells (Nos. 1, 3, 4, 7, 10 and 12) had higher values, which were above the permissible limit of 250 mg/l [17, 23, 35]. Sulphate concentration in groundwater of coastal zone were within the permissible values recommended by WHO [35], EEC [23] and ISI [17]. The major cations and anions were further analysed based on Hill-Piper trilinear diagram.

Hill-Piper diagram

Pattern diagram was initially conceived by Hill [16] and later improved by Piper [27] and the detailed analysis of Hill-Piper trilinear diagram for post monsoon season (*Fig. 2*) is explained below using facies diagram.

The hydrochemical pattern diagram helps in hydrogeochemical facies classification [5]. The trilinear diagram of this study is classified into four hydrochemical facies based on the dominance of different cations and anions: facies 1: Ca^{2+}-Mg^{2+}-HCO_3^- type I; facies 2: Na^+-K^+-Ca^{2+}-HCO_3^- type II; facies 3: Na^+-K^+-Cl^--SO_4^{2-} type III and facies 4: Ca^{2+}-Mg^{2+}-Cl^--SO_4^{2-} type IV.

Fig. 2 shows that the majority of samples were in type II (Na^+-K^+-Ca^{2+}-HCO_3^-) followed by type III (Na^+-K^+-Cl^--SO_4^{2-}) and type I (Ca^{2+}-Mg^{2+}-HCO_3^-). This indicates that post monsoon samples are enriched with sodium, bicarbonate and chloride types and, from this it is evident that sea water and tidal channel/canals plays a major role in controlling the groundwater chemical composition in the coastal shallow aquifer, which consists of recent alluvium. Nageswara [26] conducted study on groundwater salinity of the shallow aquifers in the central Kerala and inferred that salt-water encroachment into shallow aquifers can be minimised by construction of tidal barriers. The removal of sodium ions from seawater which has infiltered into fresh water aquifer has been described by a number of workers by the method of ion exchange [28, 31]. Sodium ion present in seawater will exchange to Ca^{2+} ions. The conversion of calcium bicarbonated water to sodium bicarbonate water in many aquifers is also undoubtedly due to ion exchange [4, 13]. The freshwater will change into $NaHCO_3$ type water [3]. Further, the trilinear diagram (*Fig. 2*) revealed that dug wells (Nos. 1, 3, 4, 7, 10 and 12) falling in facies 3 showed the saline water intrusion of coastal aquifers with high percentage of sodium and chloride.

Escherichia coli

The bacteriological content is one of the most important aspects in drinking water quality. The most common and widespread health risk associated with drinking water is the bacterial contamination caused either directly or indirectly by human or animal excreta. *E. coli*, a typical fecal coliform is selected as an indicator of fecal contamination. The present study revealed a high incidence of fecal coliform, which ranged 93 to 460 MPN index FC / 100 ml (*Table 1*), indicating poor sanitary condition and improper waste disposal. The seepage of *E. coli* is easier in the sedimentary formation compared to hard rock terrains [15], which supported the present study. The fecal contamination is mainly due to improper solid waste disposal from farmyard into the soak pits located very near to drinking water wells, which do not have any protecting wall [34]. According to Woods [34], effluents from point-like sources such as septic tanks and general farmyard wastes are considered as the main sources of contamination of groundwater. The lack of protecting walls will lead to the entry of contaminated runoff water into the well from the upstream. Rojas *et al.* [32] have studied the contamination of the waters of River Rimac, Peru, and the adjoining groundwater and found that the cause of pollution is due to mining and agricultural activities as well as domestic fecal pollution upstream. The presence of *E. coli* in groundwater indicates potentially dangerous situation, and requires immediate attention.

Conclusion

Analysis of groundwater samples from the study area indicated signs of deterioration, which highlights the need for a sustainable utilization of precious resources. Groundwaters present in the shallow aquifers of some of the stations were poor in quality and beyond potable limit as per the standard set by WHO and ISI. Samples from rest of these zones indicated that the groundwater quality is satisfactory (geochemically) but requires attention, with a thrust on proper sanitation and waste disposal of the adjacent coastal region. The groundwater collected from the six dug wells indicated that there is a mixing of fresh and saline water during post monsoon. The study revealed that these wells need more controlled withdrawal of water with more

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recharging in order to maintain fresh-saline water equilibrium. Further, it stressed that the coastal zone of the study area need more attention in order to maintain the ground water quality. The study also recommends the necessity of proper sanitation and waste disposal to sustain the groundwater quality.

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