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## INFLUENCE OF LASER STIMULATION SEEDS ON GERMINATION AND INITIAL GROWTH OF SEEDINGS *SILENE VULGARIS*

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**Key words:** *Silene vulgaris*, ecotype, morphological properties, laser light, seed germination, germination energy, seed germinability.

### Abstract

The aim of research was to determine the effect of pre-sowing stimulation of seeds from selected ecotypes of *Silene vulgaris* with semiconductor laser beams on increased phytoremediating ability of plants. Three ecotypes of *Silene vulgaris*, originating from the area of natural (Gajków ecotype) and elevated content of heavy metals (Wiry and Szopienice ecotypes) were subjected to investigation. The following doses of laser light were applied:  $D_1$  – single,  $D_3$  – threefold,  $D_5$  – fivefold,  $D_7$  – sevenfold – and  $D_{10}$  – tenfold irradiation with basic dose amounting  $2.5 \cdot 10^{-1} \text{ J cm}^{-2}$  and control variant  $C$  – seeds not subjected to irradiation. The ecotypes subjected to examination did significantly differ in their properties. It was possible to observe diverse response of the ecotypes to applied pre-sowing laser radiation. The improvement in the sowing value was obtained after application of doses  $D_1$ ,  $D_3$  and  $D_5$ , while stimulation of biometric traits of seedlings resulted from the introduction of doses  $D_3$ ,  $D_7$  and  $D_{10}$ . The two of them (Wiry and Gajków ecotypes) showed increased sowing value, as well as elongation of embryonic root and above – ground parts of seedlings. The ecotype originating from Szopienice proved to be insensitive to pre-sowing application of laser radiation

### WPLYW STYMULACJI LASEROWEJ NASION NA KIELKOWANIE I POCZĄTKOWY WZROST SIEWEK *SILENE VULGARIS*

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**Słowa kluczowe:** *Silene vulgaris*, ekotyp, cechy morfologiczne, światło lasera, kielkowanie nasion, energia kielkowania, zdolność kielkowania.

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### Abstract

Celem badań było określenie wpływu przedsiewnej stymulacji nasion *Silene vulgaris* promieniami lasera półprzewodnikowego na wartość siewną oraz cechy morfologiczne siewek. Stymulacja prowadziła do zwiększenia zdolności fitoremediacyjnych wybranych ekotypów. Badano trzy ekotypy *Silene vulgaris* pochodzące z obszarów o naturalnej (ekotyp Gajków) i podwyższonej zawartości metali ciężkich (ekotypy Szopienice i Wiry). Zastosowano następujące dawki światła laserowego:  $D_1$  (jedno-),  $D_3$  (trzy-),  $D_5$  (pięcio-),  $D_7$  (siedmio-) i  $D_{10}$  – dziesięciokrotne naświetlanie dawką podstawową wynoszącą  $2.5 \cdot 10^{-1} \text{ J cm}^{-2}$ , czas naświetlania 4.1 min. oraz wariant kontrolny ( $C$ ) – nasiona niepoddane naświetlaniu. Ekotypy różniły się istotnie pod względem badanych cech. Obserwowano zróżnicowaną reakcję na zastosowane przedsiewnie promieniowanie laserowe. Poprawę wartości siewnej otrzymano po zastosowaniu dawek  $D_1$ ,  $D_3$  i  $D_5$ , natomiast stymulację cech biometrycznych siewek pod wpływem  $D_3$ ,  $D_7$  i  $D_{10}$ . Dwa z badanych ekotypów (Wiry i Gajków) reagowały podwyższeniem wartości siewnej, jak również wydłużeniem korzeni zarodkowych i nadziemnych części siewek. Ekotyp pochodzący z Szopienic okazał się niewrażliwy na przedsiewne zastosowanie promieniowania laserowego.

## Introduction

Plants growing on soils containing natural high contents of heavy metals have been the object of scientists interest for a long time. However, this interest has been focused on hyperaccumulators, i.e. species absorbing and storing heavy metals in considerable amounts in relation to their total weight (PROCTOR 2003, CHANEY et al. 2005, KAZAKOU et al. 2010). On the area of Poland there do not occur typical hyperaccumulators, yet some of native species characterize high tolerance to harmful effect of heavy metals. These species are called metalophytes. To this „group” of Polish metalophytes is classified *Silene vulgaris* (*Caryophyllaceae*) (WIERZBICKA and PANUFNIK 1998, KOSZELNIK-LESZEK 2012), growing on the areas secondarily enriched in heavy metals, as well as on soils featuring natural high content of heavy metals, such as Zn – Pb ore areas, ore or serpentine areas (WIERZBICKA and PANUFNIK 1998, KOSZELNIK-LESZEK 2012, ŻOŁNIERZ 2007, NADGÓRSKA-SOCHA et al. 2011). Unique adaptation abilities of this species (BRATTELER et al. 2002) resulted in the development of separate ecotypes, capable of growing in extremely unfavorable conditions of their habitat. Apart from *S. vulgaris* ecotypes, resistant to lead (WIERZBICKA and PANUFNIK 1998, NADGÓRSKA-SOCHA et al. 2011, KANDZIORA et al. 2007), there are also known the ones which tolerate excessive amount of cadmium, zinc, copper (NADGÓRSKA-SOCHA et al. 2011, HARMENS et al. 1993, VERKLEJI and PRAST 1989) or arsenic and cobalt (PALIOURIS and HUTCHINSON 1990). Tolerance to Ni has been described as the example of non – specific co – tolerance of *S. vulgaris* to different metals (PALIOURIS and HUTCHINSON 1990, GABBRIELLI et al. 1990, WESTERBERGH 1994). Metalophytes, including *S. vulgaris*, are natural phytoremediators, used in the methods of soil remediation. This way of biological reclamation makes use of the proper-

ties of some plant species regarding absorption and accumulation of pollutants in their tissues, in the amounts exceeding those commonly found in tissues of other plants. The success of phytoremediation depends, first of all, on the choice of appropriate plant species. Therefore, they can prove to be especially valuable e.g. populations of *S. vulgaris*, whose natural resistance enables their growth in difficult habitat conditions, connected with excessive quantities of metals. Majority of naturally occurring phytoremediators are plants with poor weight gain, which are not always suitable to be widely used in a particular phytoremediatory method and, therefore, there was undertake research in order to determine the effect of pre-sowing stimulation of seeds of *Silene vulgaris* ecotypes with semiconductor laser beams on increased phytoremediatory abilities of the seedlings. Usefulness of plant stimulation with laser can be confirmed by apparent increase in plant biomass, acceleration of plant growth, as well as its influence on physiological processes (SACAŁA et al 2012, DANAILA-GUIDEA et al. 2011, PROŚBA-BIAŁCZYK et al. 2012), and increased content of biogenic elements in plant biomass (ŚLIWKA and JAKUBIAK 2009, ASHRAFIJOU et al. 2010, ŚLIWKA and JAKUBIAK 2010). Moreover, there were observed differences in accumulation of Cu, Cd, Ni in willow leaves, as well as lack of negative effect of these chemical elements on plants as their content increases (GRYGIERZEC and GOWIN 2010, JAKUBIAK and ŚLIWKA 2010).

## Materials and Methods

### General characteristics of selected ecotypes of *Silene vulgaris*

**Gajków ecotype** comes from natural habitat, from the village Gajków situated on south – east of Wrocław (Poland – Lower Silesia), (KOSZELNIK-LESZEK and WALL 2009).

**Szopienice ecotype** grows on the area situated 250 meters from pollutants emitter, i.e. Non – ferrous Metals Smelter „Szopienice” in Katowice (Poland – Upper Silesia) (NADGÓRSKA-SOCHA and CIEPAŁ 2009).

**Wiry ecotype** covers a small heap connected with exploitation of serpentine deposits in Lower Silesia near the village Wiry, located not far from western foothills of the Ślęża Mountain (KOSZELNIK-LESZEK 2007).

**Analysis of seeds of selected *Silene vulgaris* ecotypes.** The sizes of 25 randomly selected seeds from each of three *S. vulgaris* ecotypes were measured. The measurement [mm] was done in the widest and the narrowest site of each seed, using the microscope Axioskopu 2 plus, at magnitude 10x and program AxioVision 2.0.

**Laboratory experiment with the use of laser radiation.** The purpose of laboratory research was determination of the effect of treatment seeds with semiconductor laser beams on sowing value, as well as morphological character of seedlings originating from the examined ecotypes. Before establishing the experiment, seeds were exposed to laser light – semiconductor laser model CTL – 1106 MX. Applying the scanner (model CTL 1202 S), which cooperated with laser, there was determined radiation – exposed surface. There were applied the following doses of laser light:  $D_1$  – single,  $D_3$  threefold,  $D_5$  – fivefold,  $D_7$  – sevenfold and  $D_{10}$  – tenfold irradiation with basic dose amounting  $2.5 \cdot 10^{-1} \text{ J cm}^{-2}$ , exposure time 4.1 min. and control variant  $C$  – seeds not subjected to irradiation. Seeds of control and irradiated with laser light were sown in the first day after exposure to plastic plates. The experiment was established according to the method of independent series. Sowing material of *S. vulgaris* was placed in a germination apparatus in controlled temperature and humidity conditions. There were estimated energy and germination capacity – according to International Rules for Seed Testing ISTA 2007. The measurements of morphological properties of seedlings grown from control and irradiated seeds included: the length of embryonic root, above – ground parts of seedlings and cotyledons.

The results obtained due to the measurements underwent statistical analysis according to the methodology appropriate for two – factorial laboratory experiment. The subject of the assessment were: significance of ecotypes diversity, doses of laser light, as well as interaction between these factors. Test F was used in order to determine significance of differences between the variants applied, while Duncan test was applied to form homogeneous groups.

## Results and Discussion

The results of measurements of three selected seed *S. vulgaris* ecotypes are shown in Figure 1. The seeds have a natural population size which is a typical length intervals of 1.25 – 1.5 mm and a width of 2–1.25 mm and width 2–1.25 mm (MATUSZKIEWICZ 2008). The length of seeds from population coming from the areas polluted with heavy metals can be found within the lower limit of typical interval length, while seeds width are placed below lower limit model width of *S. vulgaris* seeds. Seeds of *Silene vulgaris* (Figure 2) natural population were larger than those coming from the areas of elevated content values of heavy metals and, therefore, they were probably richer in nutrients. *S. vulgaris* populations, growing in habitats contaminated with metals, developed seeds of lower, probably poorer in nutrients.

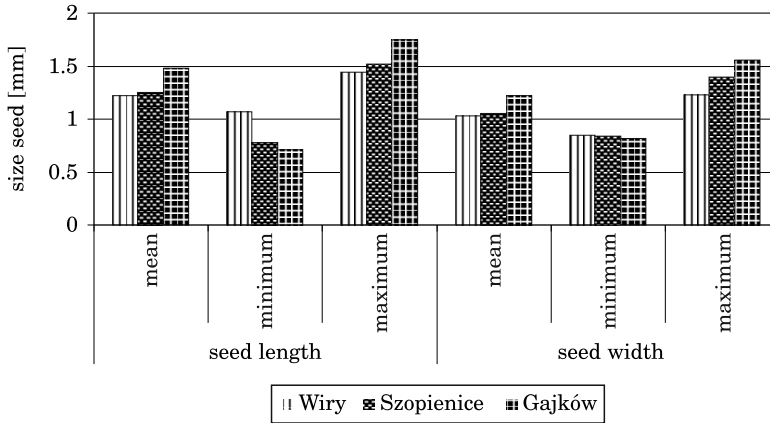


Fig. 1. Size seeds of selected ecotypes of *Silene vulgaris* [mm]

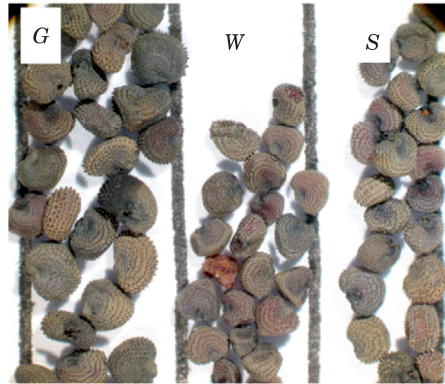


Fig. 2. Seeds of selected ecotypes of *Silene vulgaris*: G – Gajków, W – Wiry, S – Szopienice (microscope NIKON SMZ800)

Analysis of variance, applied to germination capacity, showed significant diversity of the examined ecotypes (Gajków, Wiry, Szopienice), as far as laser radiation doses were concerned. Sowing material of three investigated ecotypes did significantly differ in germination capacity. The highest value of this property belonged to the ecotype from Wiry (76.78%), lower value characterized the ecotype from Szopienice (49.61%), while the lowest one featured Gajków ecotype (41.56%) – Figure 3. Stimulating effect of pre-sowing seeds irradiation with laser beams was observed after application of dose  $D_5$  (65.89%) as compared to control amounting 59.67%. Doses  $D_{10}$  and  $D_7$  proved to be too high and resulted in reduction of the value of that property to 46.89 and

44.33% respectively (Figure 3). Interaction ecotype x dose allowed to state that the effect of germination capacity reduction in Gajków ecotype after introduction of doses  $D_1$  (40.00%),  $D_{10}$  (30.00%) and  $D_7$  (22.67%) in relation to control (56.67%). The response of Wiry ecotype to application of pre-sowing irradiation with doses  $D_5$ ,  $D_3$  and  $D_1$  was stimulation – increase in the value of the mentioned property by 23.34; 18 and 14.67% respectively in comparison to germination capacity of control seeds, which ranged 71.33%. The ecotype from Szopienice did not show any response to irradiation with laser beams (Figure 3). Considering the length of embryonic root, conducted analysis of variance demonstrated significant diversity of the examined ecotypes, as well as interaction ecotype x dose. Significantly longer embryonic root characterized ecotypes: Gajków (42.60 mm) and the one originating from Wiry dump (41.82 mm), as compared to the ecotype from Szopienice (29.34 mm) – Figure 4.

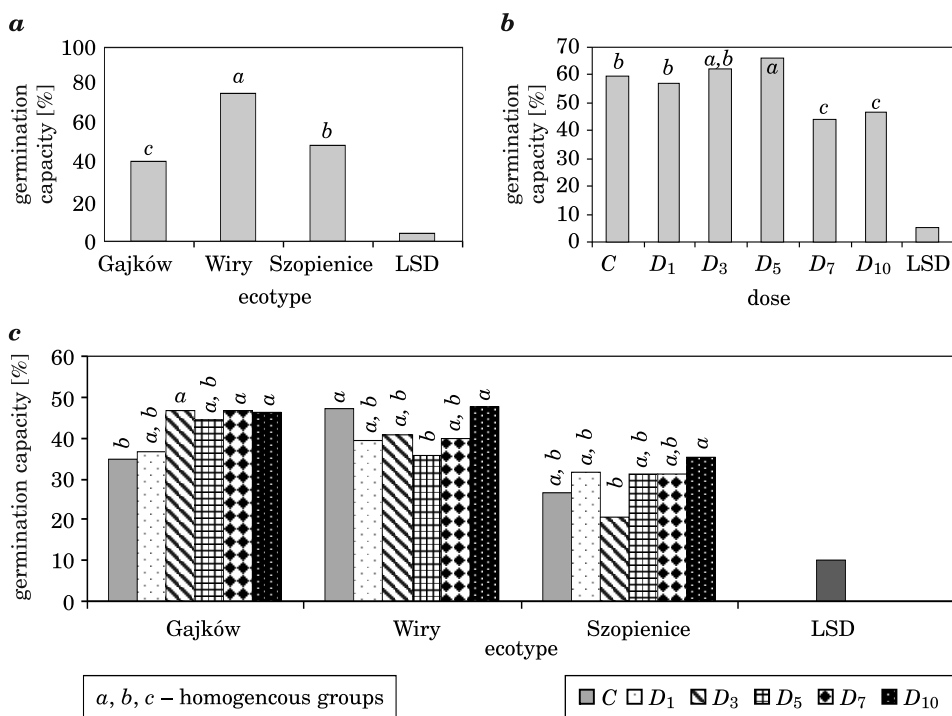


Fig. 3. Effect of stimulation of selected ecotypes of *Silene vulgaris* seeds on the germination capacity:  $a$  – ecotype;  $b$  – dose;  $c$  – interaction ecotype x dose;  $LSD_{\alpha=0.05}$

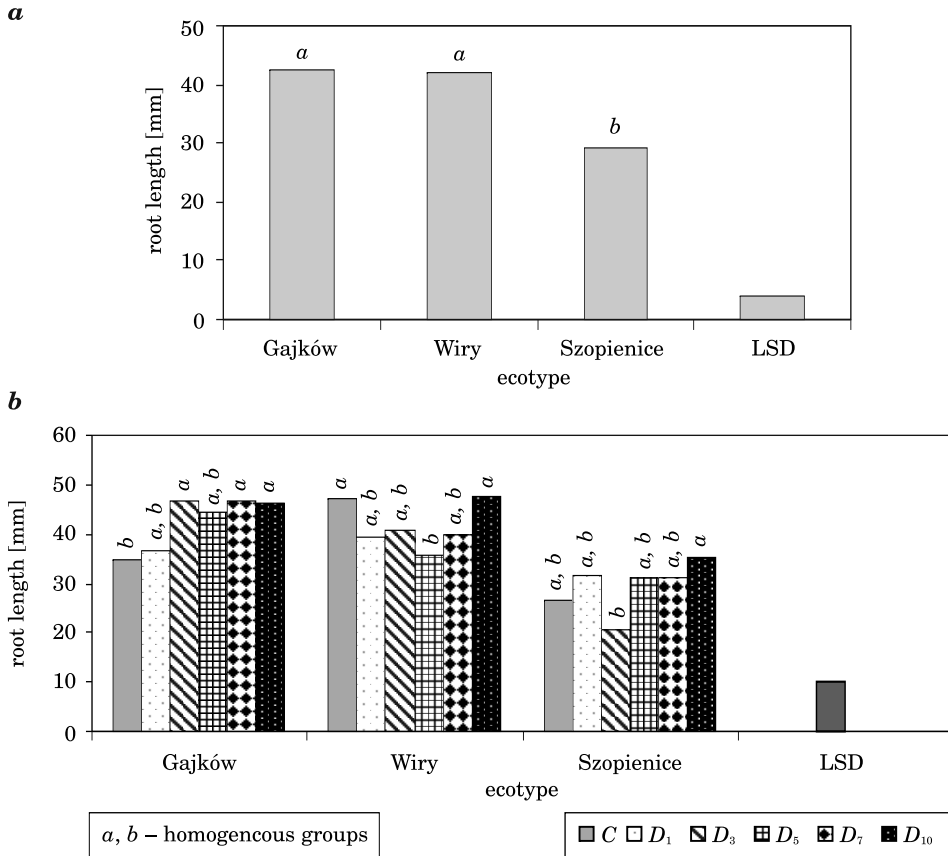


Fig. 4. Effect of stimulation of selected ecotypes of *Silene vulgaris* seeds on the embryonic root length: a – ecotype; b – interaction ecotype x dose; LSD $_{\alpha=0.05}$

Interaction proved the effect of stimulation in Gajków ecotype after application of three-, seven and tenfold irradiation (46.73; 46.73 and 46.17 mm respectively) in comparison to the length of embryonic root developed from control seeds (34.8 mm). Wiry ecotype responded by reducing the length of embryonic root after application of dose  $D_5$  by 11.47 mm in relation to control.

The ecotype coming from Szopienice, as in the case of its germination capacity, did not respond to pre-sowing exposition of seeds to semiconductor laser beams (Figure 4). Analysis of variance, conducted for the length of above-ground parts of a seedling, proved significant diversity of laser irradiation doses, ecotypes, as well as interaction ecotype x dose. The examined *S. vulgaris* ecotypes significantly differed in the length of seedlings. The longest seedlings were produced by natural ecotype Gajków (9.03 mm), the second longest was Wiry ecotype (8.18 mm), while the shortest seedlings characterized the ecotype

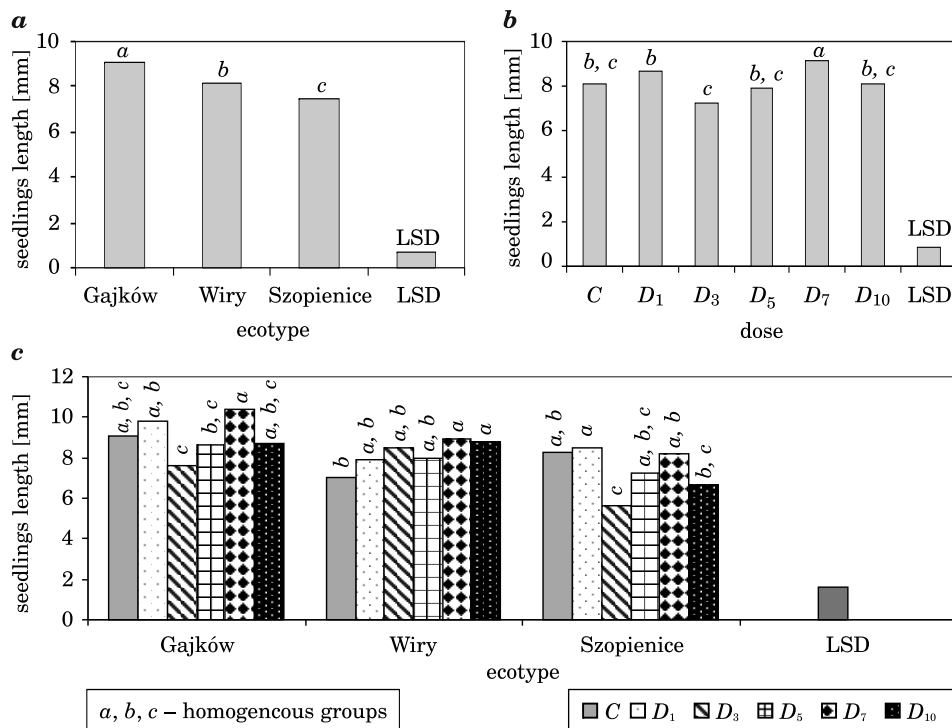


Fig. 5. Effect of stimulation of selected ecotypes of *Silene vulgaris* seeds on seedlings length: a – ecotype; b – dose; c – interaction ecotype x dose; LSD $_{\alpha=0.05}$

originating from Szopienice – 7.42 mm (Figure 5). Among the doses applied, only sevenfold irradiation of seeds resulted in the effect of stimulation, causing development of seedlings measuring 9.17 mm in length as compared to control seedlings which were 8.12 mm long (Figure 5). On the basis of interaction ecotype x dose it was possible to state the effect of stimulation in Wiry ecotype after introduction of doses D<sub>7</sub> and D<sub>10</sub> (8.9 and 8.8 mm), while control produced seedlings 7.0 mm long. The ecotype from Szopienice showed reduction in above – ground part of seedlings by 2.7 mm and ecotype A did not show any response to pre-sowing irradiation (Figure 5). As far as the length of cotyledon was concerned, analysis of variance showed significant differences in laser irradiation doses, ecotypes and interaction ecotype x dose. The longest cotyledon featured Gajków ecotype (7.43 mm). Wiry ecotype produced cotyledon of 6.86 mm in length, while significantly shortest one was developed by Szopienice ecotype (6.27 mm) – Figure 6. Among diversified doses of laser radiation only sevenfold irradiation caused the response on the side of the examined ecotypes. Dose D<sub>7</sub> resulted in significant elongation of cotyledon – 8.49 mm in relation to cotyledons produced by control seedlings – 6.82 mm



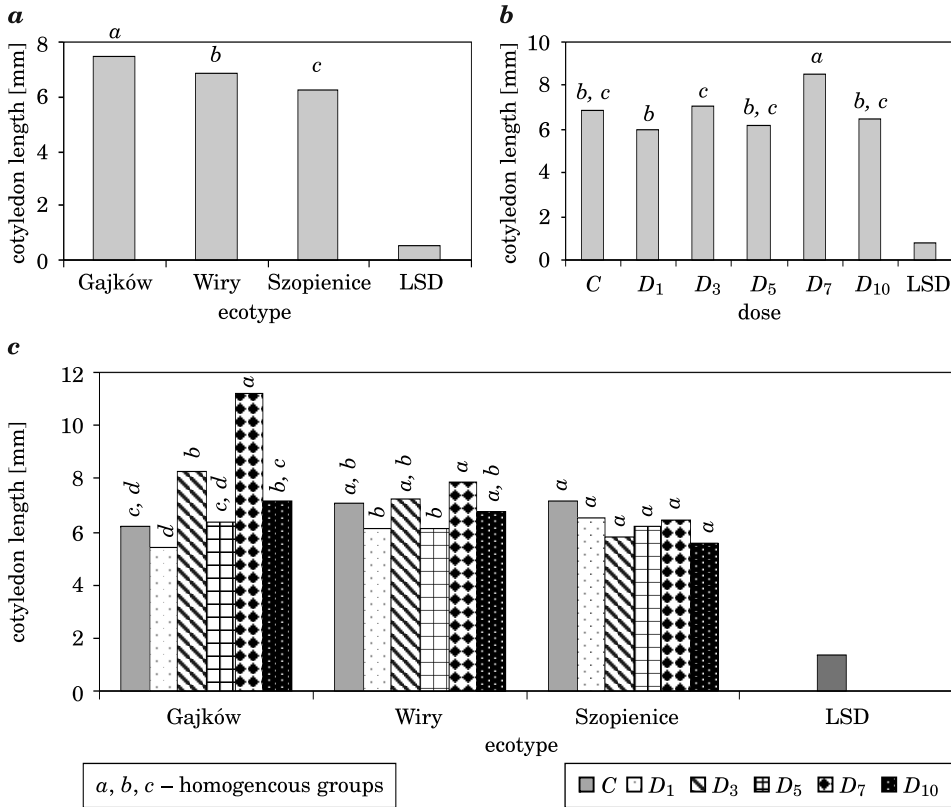


Fig. 6. Effect of stimulation of selected ecotypes of *Silene vulgaris* seeds on cotyledon length: a – ecotype; b – dose; c – interaction ecotype x dose; LSD $_{\alpha=0.05}$

(Figure 6). Considering three examined ecotypes, of *S. vulgaris* in terms of the length of cotyledons, only „Gajków” ecotype showed the effect of stimulation. After application of seven – and threefold irradiation cotyledons became elongated by 4.97 and 2.04 mm respectively in relation to control seedlings cotyledons (6.23 mm). The remaining ecotypes (Wiry and Szopienice), subjected to the research, did not show any response to the use of pre-sowing irradiation of seeds with semiconductor laser beams (Figure 6). In the studies, in selected genotypes of cereals, a significant increase in energy and germination after the introduction of pre-sowing laser irradiation was observed. Stimulation of morphological traits such as seedling root length of embryonic root, coleoptiles and aboveground parts of the seedlings were recorded after application of three- and five-time irradiation (SZAJSNER 2009). Research conducted by PODLEŚNY (2000, 2002) and PODLEŚNY et al. (2012) showed

positive effects of pre-sowing laser stimulation on the growth and development of lupine and faba bean seedlings. Similar effects were obtained by SZAJSNER et al. (2013) and PROŚBA-BIAŁCZYK et al. (2013) in studies on the effects of laser radiation on the sugar beet seeds.

Research by ŚLIWKA and JAKUBIAK (2009, 2010) on laser stimulation of plants, confirmed statistically significant increase in duckweed and yellow iris biomass in the first and in the subsequent years of conducting experiment, without any necessity of re-exposure of plant material to laser light. Stimulated plants characterized considerable resistance to decrease in temperature, as well as higher survival rate in subsequent years of the experiment. In varieties of energy willows there was also observed increased biomass of leaves after irradiation with laser diode (JAKUBIAK, ŚLIWKA 2008). Exposure to coherent light did significantly influence on increase in roots biomass, their length and density in hydroponic cultivation in salt solutions, which proves acceleration of the process of rhizogenesis (JAKUBIAK, ŚLIWKA 2009).

## Conclusions

Conducted research and observation undertaken in the course of breeding of selected *Silene vulgaris* ecotypes allow to draw the following conclusions:

1. *Silene vulgaris* plants of serpentine dump (Wiry ecotype and Zn – Pb ore Szopienice ecotype) produce smaller seeds in comparison to seeds of Gajków natural ecotype.

2. Application of dose  $D_5$  caused increase in germination capacity by 6.22%, while the dose  $D_7$  resulted in the elongation of the aboveground parts of the seedlings, as well as significant increase in the cotyledons in the studied ecotype.

3. After seeds irradiation with doses  $D_3$ ,  $D_7$  and  $D_{10}$ , embryonic root elongation in natural Gajków ecotype was recorded. Significantly longer cotyledons were observed in this ecotype under the influence of  $D_7$  and  $D_3$  doses.

4. Improved germination in Wiry ecotype was observed after application of doses  $D_1$ ,  $D_3$  and  $D_5$ , while under the influence of dose  $D_7$  and  $D_{10}$  a significantly longer seedling was produced.

5. Pre-sowing application of laser radiation did not cause any effects in Szopienice ecotype.

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**ASSESSMENT OF OCCURRENCE MICROPHYTES  
AND TROPHIC STATUS OF A SMALL WATER BODY  
IN THE WIELKOPOLSKA REGION  
(WESTERN POLAND)**

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**Key words:** water body, phytoplankton, trophic state index, chlorophyll *a*.

**Abstract**

The paper presents results of studies on microphytes found in a small and shallow water body located in the village of Drwesa in the Dopiewo community (near Poznań). The aim of investigations conducted in 2011 was to determine the taxonomic composition, abundance and biomass of microphytes, the amount of seston and selected environmental factors. The greatest species richness was observed for green algae and diatoms, while the total abundance of microphytes was usually moderate. Flagellates predominated, mainly chrysophytes, dinoflagellates and cryptophytes. Maximum abundance were recorded in spring and their frequent dips in summer and autumn. Microphyte biomass estimated by the concentration of chlorophyll *a* was generally high and significantly correlated with the amount of seston. Indicator species comprised 35% of the microphytes taxa and eutrophic indicator species predominat. The structure of microphytes indicated mesotrophy of the water body, while the concentrations of chlorophyll *a* and seston showed eutrophy.

**OCENA WYSTĘPOWANIA MIKROFITÓW A STAN TROFII MAŁEGO ZBIORNIKA  
WODNEGO W WIELKOPOLSCE (ZACHODNIA POLSKA)**

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**Słowa kluczowe:** zbiornik wodny, fitoplankton, indeks trofii, chlorofil *a*.

## A b s t r a k t

W pracy przedstawiono wyniki badań mikrofitów występujących w małym i płytkim zbiorniku wodnym położonym w miejscowości Drwęża w gminie Dopiewo (w sąsiedztwie Poznania). Badania przeprowadzono w 2011 r., a celem ich było określenie składu taksonomicznego, liczebności, biomasy mikrofitów, ilości sestonu oraz wybranych czynników środowiskowych. Największym bogactwem gatunkowym charakteryzowały się zieleńce i okrzemki, natomiast liczebność ogólna mikrofitów była na ogół umiarkowana. Stwierdzono dominację form wiciowych, głównie złotowiciowców, bruzdnic i kryptofitów. Maksimum liczebności mikrofitów zaobserwowano wiosną, natomiast latem i jesienią często się ona zmniejszała. Biomasa mikrofitów wyrażona koncentracją chlorofilu *a* była na ogół wysoka i skorelowana istotnie z ilością sestonu. 35% taksonów mikrofitów stanowiły gatunki wskaźnikowe, przeważały wśród nich wskaźniki eutrofii. Struktura mikrofitów wskazała na mezotrofię wód badanego zbiornika, natomiast koncentracja chlorofilu *a* i sestonu na eutrofię.

**Introduction**

Two basic groups of primary producers are distinguished in water bodies, i.e. small, suspended in the pelagic zone – the so-called phytoplankton (microphytes), as well as large, connected typically with the littoral zone, i.e. hydromacrophytes. Both these groups are commonly used in water quality assessment. Hydromacrophytes are relatively permanent components, undergoing relatively slow changes, while microphytes are characterized by a rapid response to changing environmental conditions due to their short life cycles. This makes microphytes one of the most dynamic groups in the ecosystem, while due to their key importance as primary producers is also one of the most frequently studied (HUTCHINSON 1957, MCCORMICK and CAIRNS 1994).

Research on microphytes concern both the species composition and their population abundance as well as biomass. Much information on their communities is used in the assessment of the quality of waters. It is commonly believed that with an increase in trophic status of the water body the diversity of algae and cyanobacteria decreases and their density and biomass increase. However, in many cases species richness and diversity of microphytes are quite unrelated to trophic state and productive capacity (KAWECKA and ELORANTA 1994, DODSON et al. 2000). Some species of aquatic microorganisms are representative of the oligotrophic waters while others are typical of the eutrophic habitats (REYNOLDS 1984, RAKOWSKA et al. 2005).

The aim of this study was to assess the trophic status of a small water body located in the rural area on the bases of the species composition and abundance of microphytes in connection with selected environmental factors. It was tested how different indicators describe trophic status this water body. Analyses concerned the number of taxa, the occurrence of indicator species for individual trophic states and density of organisms as well as concentration of chlorophyll *a*. Pond in Drwęża in this aspect was not considered until now,

despite its great recreational importance. Moreover, selected physical and chemical parameters of water were presented. Relationships between them and microphytes were determined.

## Study area

The examined pond is located in the Dopiewo community in the village of Drwęsa, approx. 20 km west of Poznań (Figure 1). In terms of the physico-geographical division of Poland into regions (KONDRACKI 1998) this area belongs to the Central Polish Lowland, Macroregion – the Wielkopolska Lake District, Mesoregion – the Poznań Lake District, Microregion – the Owińska-Kiekrz Hills. The analysed pond is very small and relatively shallow. It is only 0.03 ha in area with the maximum depth of 1.9 m. The lake basin is oval and the shoreline is relatively uniform. Only in the northern part there is a small cove. The shoreline is 62 m in length. The maximum width of the pond is 16.5 m, while the maximum length is 23 m. The water level of the pond is at 88.07 m a.s.l.

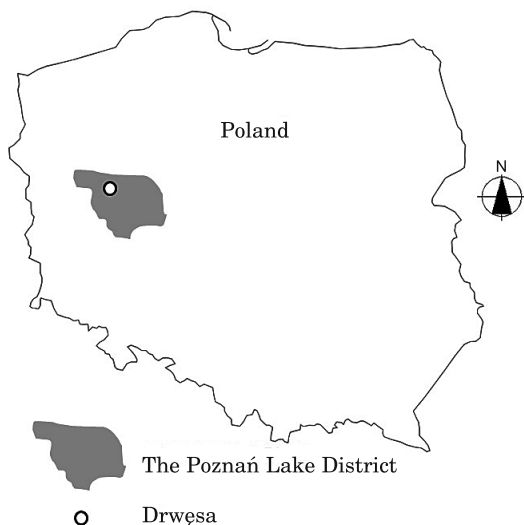


Fig. 1. Location of the pond in Drwęsa

The pond in Drwęsa is of anthropogenic origin, as it was formed as a result of deepening of a limited depression in the late 1980's, in which water accumulated after heavy rains. Rainwater from roofs of the neighbouring buildings with the total area of 380 m<sup>2</sup> was discharged to the northern part of

the water body through a system of pipes. The water body is characterized by a variable water level. In spring the water level is as a rule the highest, while in the other seasons of the year it is gradually reduced.

The macrophytes in the study period included *Typha latifolia* L. that formed patches in the north-eastern part of the pond as well as in the small cove. At the peak of the vegetation season *Ceratophyllum demersum* L. predominated, filling almost entirely the pond basin, and it was accompanied by *Lemna minor* L., *Lemna trisulca* L. and filamentous algae.

## Materials and Methods

Water samples were collected in 2011: in winter and autumn once a month, while in spring and summer biweekly. Samples were collected from the surface water layer in the centre of the water body for analyses of plankton, the concentrations of chlorophyll *a* and seston. Samples for plankton analyses were fixed with Lugol's iodine with sodium acetate (STARMACH 1963). When collecting samples, measurements for water temperature, pH and electrolytic conductivity were taken using a HANNA meter.

Spectrophotometry corrected for phaeopigments was used to measure chlorophyll *a*, in accordance with the Polish Standard. Seston dry mass was determined by gravimetry, with it being condensed on Whatman GF/F filters. Microphyte composition and density were analysed using an inverted microscope (PZO, MOD-2) and cylindrical plankton chambers of 14 ml at a magnification of 40, 150 and 600x (WETZEL and LIKENS 1991). Microphytes were determined according to the method presented by LUND et al. (1958). For identification and nomenclature of algae and cyanobacteria Polish Flora Freshwater were used (STARMACH 1963, 1966).

Trophic state was evaluated based on indicator taxa given by HUTCHINSON (1957), HÖRNSTRÖM (1981), ROSÉN (1981), JÄRNEFELT (acc. KAWECKA and ELORANTA 1994). A three-point scale was adopted expressed in the number index, with the number 3 corresponding to eutrophy, 2 to mesotrophy, while 1 to oligotrophy (SZELAĞ-WASIELEWSKA et al. 1999, SZELAĞ WASIELEWSKA and GOLDYN 2005). Moreover, it was assumed that in the gradient of trophic state values of the index ranging from 0.00 to 0.74 corresponded to oligotrophy, within the range of 0.75 to 1.24 to oligomesotrophy, 1.25–1.74 to mesotrophy, 1.75–2.24 to mesoeutrophy, 2.25–2.74 to eutrophy, 2.74–3.00 to high eutrophy. The trophic index was calculated for the numbers of indicator taxa using the formula (HÖRNSTRÖM 1981):



$$I_c = \frac{\sum (f \cdot I_t)}{\sum f}$$

where:

$I_c$  – trophic index of the community;

$I_t$  – trophic index of given species;

$f$  – numbers of the indicator species.

Moreover, to assess the trophic state the OECD lake classification based on the criteria developed by VOLLENWEIDER (1971) and indicators of the trophic state according to CARLSON (1977) (Trophic State Index – TSI) adopting one parameter, i.e. chlorophyll  $a$  were used.

Statistical analysis of the results covered calculations of linear correlation coefficients between the investigated factors. It was performed using Excel 2007.

## Results

### Microphytes

Cyanobacteria and 8 groups of eukaryotic algae were found in the pond plankton. A total of 98 taxa were identified, with the highest number recorded for green algae (Chlorophyta) with 40 taxa, diatoms (Bacillariophyceae) with 18 and cryptophytes (Cryptophyceae) with 13, respectively. In the case of other groups (Cyanobacteria, Euglenophyceae, Dinophyceae, Xanthophyceae, Chrysophyceae) it was lower, ranging from 1 to 8. In individual months the number of taxa ranged from 10 to 28 (Table 1).

The highest number of taxa (28) was recorded in June, while the lowest was found in February, when the species richness of green algae and cryptophytes was identical. Green algae and cryptophytes occurred throughout the year, while euglenophytes, dinoflagellates, chrysophytes and diatoms appeared over a major part of the year. Representatives of cyanobacteria and xanthophytes were observed in the pelagic zone only periodically (Table 1). The number of taxa was significantly and positively correlated with the water temperature ( $r = 0.758$ ). The other analysed relations were non-significant, although they were mostly positive (Table 2).

The total abundance of microphytes fell within a wide range from  $0.78 \times 10^3$  cells  $\text{ml}^{-1}$  to  $19.5 \times 10^3$  cells  $\text{ml}^{-1}$ , with the ratio of the maximum value to the minimum value of approx. 25. In spring the abundance was much higher than in the other seasons and it exceeded as much as three-fold the value of  $10^3$  cells  $\text{ml}^{-1}$ . Most frequently the density of microphytes was changed within the range of  $2\text{--}5 \times 10^3$  cells  $\text{ml}^{-1}$ , while in the first half of the year the mean value was

Table 1  
Number of microphytes taxa in the plankton in Drwęża pond in 2011

Specification	31.1	28.2	30.3	20.4	07.5	25.5	15.6	30.6	14.7	28.7	10.8	31.8	18.9	30.9	14.10	31.10	16.11	07.12	Total
Cyanobacteria	-	-	1	-	-	1	-	-	1	-	1	-	2	2	1	-	-	-	4
Euglenophyceae	1	-	2	2	2	3	3	3	-	1	2	1	3	1	2	2	2	2	8
Cryptophyceae	6	4	3	4	3	3	2	3	4	3	5	4	6	5	5	5	4	2	13
Dinophyceae	1	1	-	-	1	-	3	3	1	2	1	3	1	1	2	1	1	1	7
Xanthophyceae	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	1
Chrysophyceae	-	1	1	5	3	3	2	3	2	1	2	-	1	-	-	1	1	1	7
Bacillariophyceae	-	-	2	5	2	7	6	2	4	3	1	4	5	7	2	1	1	2	18
Chlorophyta	4	4	6	4	5	9	12	13	12	5	3	2	7	8	6	3	3	4	40
Total	12	10	15	20	17	26	28	28	24	15	15	14	25	24	18	12	12	12	98

Table 2  
The coefficients correlation of number of taxa and microphytes abundance versus selected physical and chemical parameters of water

Specification	Water temperature	Water reaction	Conductivity	Seston dry mass	Chlorophyll <i>a</i>	Number of taxa	Microphytes abundance	<i>I<sub>c</sub></i> *
Water temperature	–	–	–	–	–	–	–	–
Water reaction	0.229	–	–	–	–	–	–	–
Conductivity	–0.037	–0.344	–	–	–	–	–	–
Seston dry mass	0.024	0.494*	0.243	–	–	–	–	–
Chlorophyll <i>a</i>	–0.016	0.398	–0.084	0.769***	–	–	–	–
Number of taxa	0.758***	0.347	0.197	0.248	0.008	–	–	–
Microphytes abundance	–0.131	–0.466*	0.715***	–0.012	–0.212	–0.009	–	–
<i>I<sub>c</sub></i> *	–0.017	–0.223	–0.068	–0.640**	–0.621**	0.066	0.043	–

\* – Trophic State Index based on the microphytes abundance of indicator species;

\*\* –  $\alpha < 0.01$ ; \*\*\* –  $\alpha < 0.001$ ).

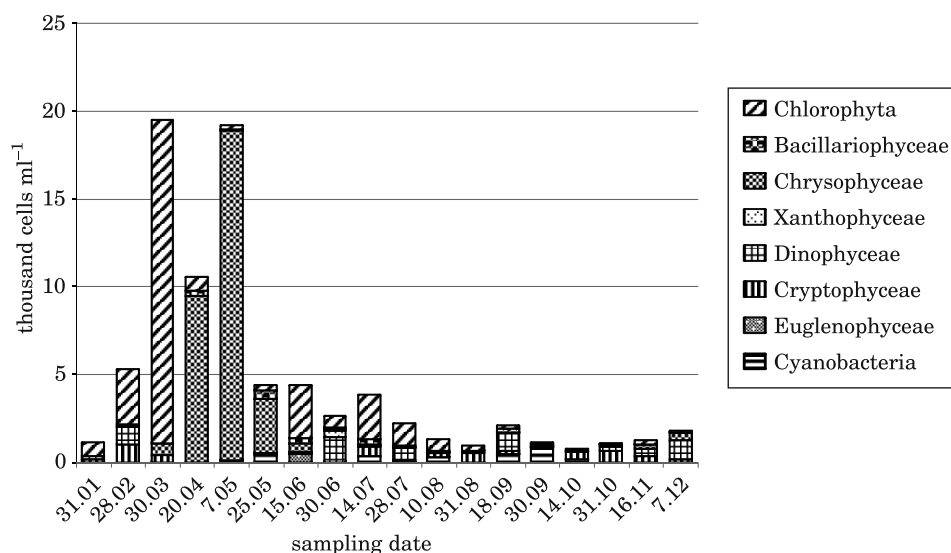


Fig. 2. Microphytes abundance in plankton in Drwesa pond during 2011

approx.  $8 \times 10^3$  cells  $\text{ml}^{-1}$  and in the second half it was almost  $2 \times 10^3$  cells  $\text{ml}^{-1}$ . In March green algae were most abundant, while in April and May it was chrysophytes. Among the other groups dinoflagellates and cryptophytes were numerous and constant components of microphytes. The contribution of

cyanobacteria, diatoms and euglenophytes to the total abundance were small, at max. 8.5%, 4% and 1.2%, respectively (Figure 2).

A strong positive correlation ( $r = 0.715$ ) was found between the total abundance of phytoplankton and electrolytic conductivity. In turn, correlation between the total abundance and water reaction was negative and weak ( $r = -0.466$ ) (Table 2). Among the identified taxa some formed highly numerous populations. The most abundant comprised *Synura uvella* Ehrenberg, *Koliella longiseta* (Vischer) Hindák, *Scourfieldia* sp., *Uroglena americana* Calkins, *Chlamydomonas* sp., *Chlorella* sp., *Spermatozopsis exultans* Koršikov, *Woloszynskia hiemalis* (Wołoszyńska) Thompson. Frequently these taxa appeared many times throughout the year of the study (Table 3).

Table 3

The most important taxa with regard to the microphytes abundance in Drwęża pond in 2011

Sampling date	Name of taxa and abundance [cells ml <sup>-1</sup> ]	
31.01.2011	<i>Chlamydomonas</i> sp. (700)	<i>Woloszynskia hiemalis</i> (182)
28.02.2011	<i>Chlamydomonas</i> sp. (2900)	<i>Woloszynskia hiemalis</i> (1040)
30.03.2011	<i>Koliella longiseta</i> (11200)	<i>Scourfieldia</i> sp. (6800)
20.04.2011	<i>Synura uvella</i> (7700)	<i>Dinobryon sociale</i> var. <i>americanum</i> (1737)
07.05.2011	<i>Synura uvella</i> (12700)	<i>Uroglena americana</i> (6000)
25.05.2011	<i>Synura uvella</i> (2986)	<i>Aphanocapsa</i> sp. (408)
15.06.2011	<i>Chlamydomonas</i> sp. (1495)	<i>Dictyosphaerium</i> sp. (848)
30.06.2011	<i>Gymnodinium uberrinum</i> var. <i>rotundatum</i> (1061)	<i>Tribonema minus</i> (364)
14.07.2011	<i>Spermatozopsis exultans</i> (1091)	<i>Chlorella</i> sp. (586)
28.07.2011	<i>Chlorella</i> sp. (1131)	<i>Gymnodinium uberrinum</i> var. <i>rotundatum</i> (424)
10.08.2011	<i>Chlorella</i> sp. (606)	<i>Aphanocapsa incerta</i> (286)
31.08.2011	<i>Cryptomonas undulata</i> (330)	<i>Chlorella</i> sp. (264)
18.09.2011	<i>Gymnodinium uberrinum</i> var. <i>rotundatum</i> (1020)	<i>Aphanocapsa incerta</i> (381)
30.09.2011	<i>Aphanocapsa incerta</i> (667)	<i>Pseudanabeaena minima</i> (152)
14.10.2011	<i>Cryptomonas ovata</i> (273)	<i>Pseudanabeaena minima</i> (162)
31.10.2011	<i>Cryptomonas rostrata</i> (323)	<i>Woloszynskia hiemalis</i> (283)
16.11.2011	<i>Woloszynskia hiemalis</i> (451)	<i>Cryptomonas rostrata</i> (273)
07.12.2011	<i>Woloszynskia hiemalis</i> (1088)	<i>Synura uvella</i> (383)

Table 4

Phytoplankton indicator taxa found in Drwęsa pond during 2011

Taxa	The authors giving a taxon	Species trophic state index	Taxa	The authors giving a taxon	Species trophic state index
<i>Aphanocapsa incerta</i>	<i>e</i>	2,5	<i>Ankistrodesmus stipitatus</i>	<i>c</i>	2,8
<i>Aphanocapsa</i> sp.	<i>a, b, c, d</i>	2,5	<i>Elakatothrix acuta</i>	<i>c</i>	1,2
<i>Oscillatoria lacustris</i>	<i>d</i>	3	<i>Kirchneriella</i> sp.	<i>a</i>	3
<i>Euglena acus</i>	<i>a</i>	2	<i>Lagerheimia wratislaviensis</i>	<i>a</i>	3
<i>Euglena</i> sp.	<i>a</i>	2	<i>Monoraphidium griffithii</i>	<i>b</i>	1
<i>Euglena texta</i>	<i>a</i>	2	<i>Oocystis</i> sp.	<i>b</i>	1
<i>Phacus longicauda</i> var. <i>pyrum</i>	<i>a</i>	3	<i>Scenedesmus acuminatus</i>	<i>a, b, d</i>	3
<i>Trachelomonas volvocina</i>	<i>b, d</i>	3	<i>Scenedesmus acutus</i>	<i>a, b, d</i>	3
<i>Gymnodinium</i> sp.	<i>d</i>	1	<i>Scenedesmus armatus</i>	<i>a, b, d</i>	3
<i>Gymnodinium uberrimum</i> var. <i>rotundatum</i>	<i>d</i>	1,1	<i>Scenedesmus brasiliensis</i>	<i>a, b, d</i>	3
<i>Kephyrion</i> sp.	<i>a</i>	1	<i>Scenedesmus obliquus</i>	<i>a, b, d</i>	3
<i>Synura uvella</i>	<i>b</i>	2	<i>Scenedesmus quadricauda</i>	<i>a, b, d</i>	3
<i>Uroglena americana</i>	<i>c</i>	1,3	<i>Scenedesmus quadrispina</i>	<i>a, b, d</i>	3
<i>Cyclotella</i> sp.	<i>c</i>	1	<i>Scenedesmus spinosus</i>	<i>a, b, d</i>	3
<i>Synedra acus</i>	<i>a, c</i>	1,7	<i>Tetraedron caudatum</i> var. <i>incisum</i>	<i>b</i>	2
<i>Synedra acus</i> var. <i>angustissima</i>	<i>a, c</i>	1,7	<i>Tetrastrum triangulare</i>	<i>a</i>	3
<i>Ankistrodesmus gracilis</i>	<i>c</i>	2,8			

*a* – Järnefelt (acc. KAWECKA and ELORANTA 1994), *b* – HUTCHINSON (1967), *c* – HÖRNSTRÖM (1981), *d* – ROSEN (1981), *e* – STARMACH (1966)

A total of 34 indicator taxa were found in microphyte communities, i.e. 34.7% of their total number, of which 56% were classified as indicators of eutrophy, 21% of mesotrophy, and 24% to indicators of oligotrophy. Within the year the value of the index changed within a wide range of values (from 1.27 to 2.59). From January to April values of the index were identical (2.0) indicating a mesotrophic character of the water body. From May this index changed dynamically with the minimum value at the end of June and the maximum value in mid-July, i.e. in the range from oligomesotrophy to eutrophy (Figure 3). Sudden changes in the value of the index might be caused by the appearance of cyanobacteria from the genus *Aphanocapsa*, green algae from the genus *Kirchneriella* or dinoflagellates from the genus *Gymnodinium*, being indicators of eutrophy or oligotrophy, respectively (Table 4). The mean water trophic state

index was 1.85, which indicates mesotrophy. Trophic state index was correlated with the dry mass of seston ( $r = -0.640$ ) and the concentration of chlorophyll  $a$  ( $r = -0.621$ ) – Table 2.

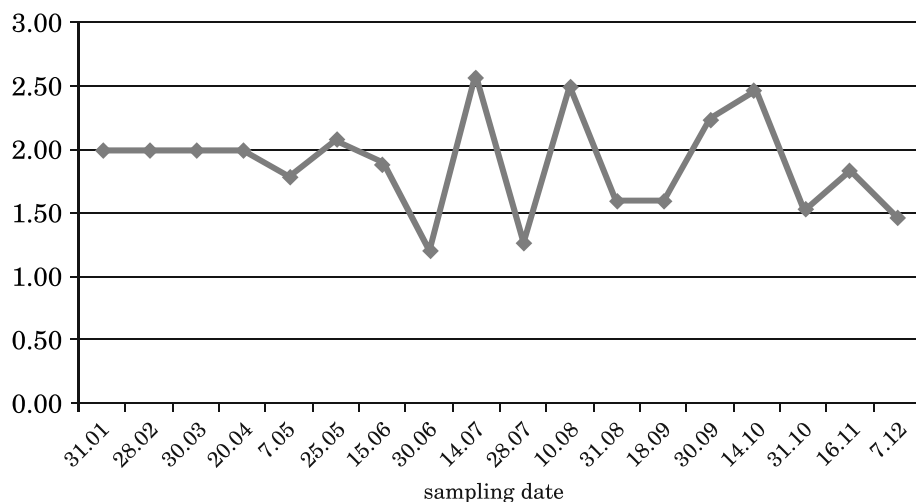


Fig. 3. Phytoplankton trophic index in Drwęsa pond in 2011

### Physico-chemical properties of water

Water temperature in the pond ranged from 0.3°C to 19.7°C. Its mean value for the entire year was 11.4°C. The highest water temperature was observed at the end of June and in mid-July, while it was lowest in February. Water reaction was usually slightly alkaline and no considerable changes were observed (7.6–8.4). At the end of June the reaction was highest, while it was lowest towards the end of August. Water reaction was significantly correlated with seston dry mass ( $r = 0.494$ ) – Table 2. In turn, electrolytic conductivity changed markedly throughout the year. Its value ranged from 480  $\mu\text{S cm}^{-1}$  to 880  $\mu\text{S cm}^{-1}$  (mean 566  $\mu\text{S cm}^{-1}$ ), while in the first half of the year it frequently exceeded 600  $\mu\text{S cm}^{-1}$ , whereas in the second half it fell within the range from 400 to 550  $\mu\text{S cm}^{-1}$  (Figure 4).

Average content of dry mass of seston was 7.3  $\text{mg l}^{-1}$ , ranging from 2.3  $\text{mg l}^{-1}$  (mid-October) to 16  $\text{mg l}^{-1}$  (end of June). Throughout the year several significant increases were found in seston dry mass (February, end of June, mid-September and early December) – Figure 5. The most significant relation with correlation coefficient  $r = 0.769$  was observed between the amount of seston and the concentration of chlorophyll  $a$ . Moreover, seston was negatively correlated with the trophic state index ( $I_c$ ) ( $r = -0.640$ ) – Table 2.

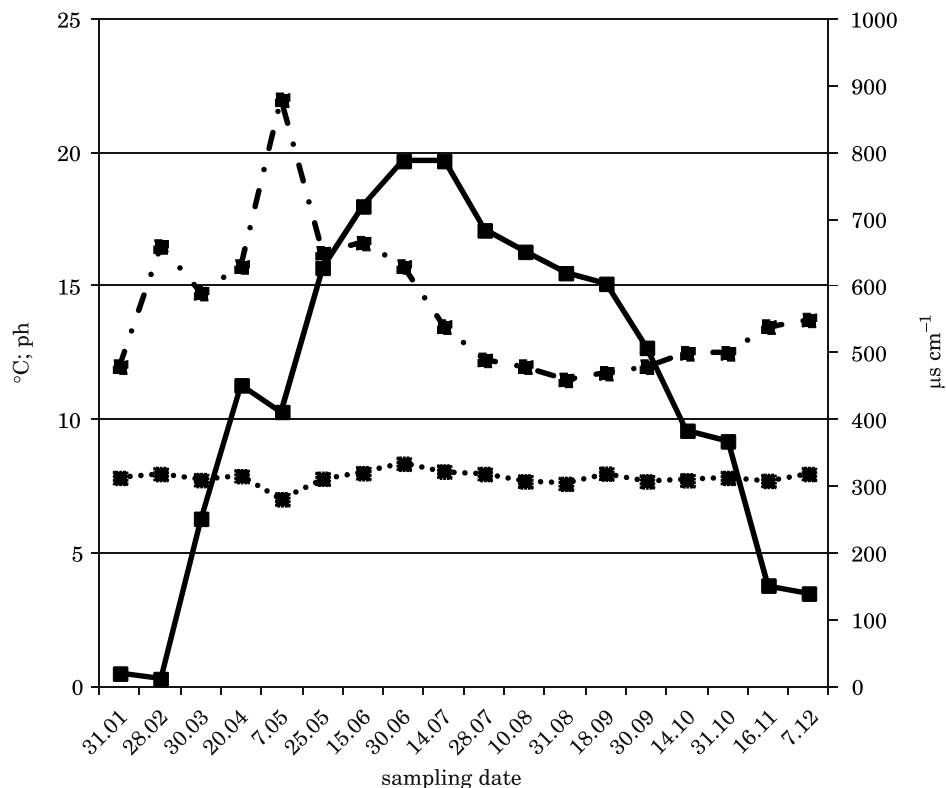


Fig. 4. Water temperature (solid line), pH (dotted line) and water conductivity (dashed line) in Drwęsa pond in 2011

Concentration of chlorophyll *a* ranged from 14.8 to 121.3  $\mu\text{g l}^{-1}$  (mean 47  $\text{mg l}^{-1}$ ). The highest value was recorded in December. A high dynamic was observed for changes in chlorophyll *a* content: in the first half of the year it was generally lower, while in the second half it was much higher, correlating significantly with the seston dry mass content (Figure 5). Concentration of chlorophyll *a* was not significantly correlated with the abundance of microphytes, but the highest values were found when abundant small in size green algae (*Chlamydomonas* sp.) or large dinoflagellates (*Gymnodinium uberrimum* var. *rotundatum*, *Woloszynskia hiemalis*) and chrysophytes (*Synura uvella*) occurred. This situation was observed e.g. in February, May, end of June, mid-September and in December (Table 3, Figure 5). Based on the maximum value of chlorophyll *a* the pond may be classified as strongly eutrophic. Also mean value for chlorophyll *a*, amounting to 47  $\mu\text{g l}^{-1}$ , indicates a high trophic state of the waters. The value of TSI based on chlorophyll *a* ranged from 57 to 78 at a mean of 66.6 (Table 5).

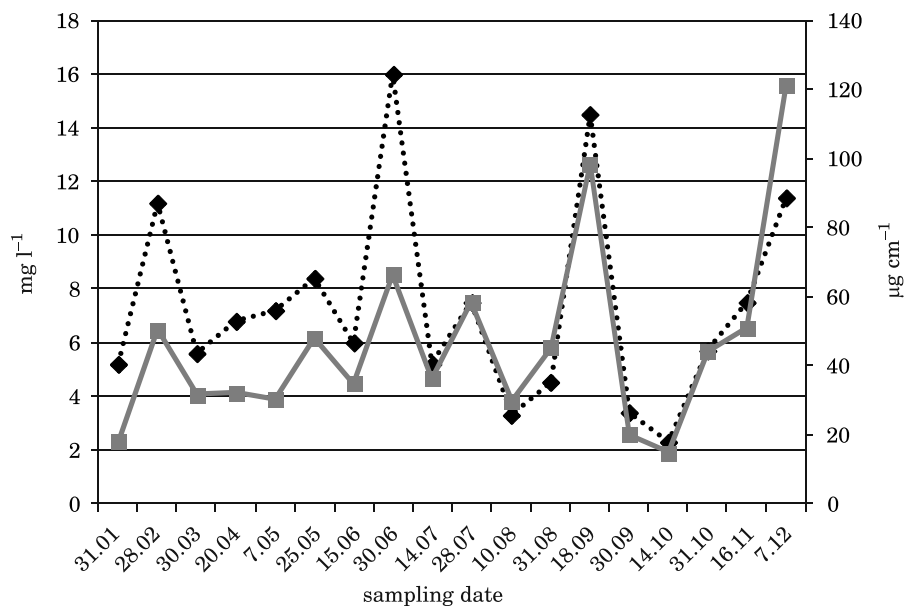


Fig. 5. Changes in seston dry mass (dashed line, mg l<sup>-1</sup>), chlorophyll *a* (solid line, µg l<sup>-1</sup>) in Drwęsa pond in 2011

Table 5  
Seasonal changes of Trophic Lake Index based on concentration of chlorophyll *a* in 2011 (CARLSON 1977)

Sampling date	Index value
31.01	58.90
28.02	69.01
30.03	63.43
20.04	64.59
07.05	64.03
25.05	68.53
15.06	65.43
30.06	71.73
14.07	65.84
28.07	70.47
10.08	63.86
31.08	67.96
18.09	75.59
30.09	59.98
14.10	56.97
31.10	67.70
16.11	69.10
07.12	77.64
Average	66.56



## Discussion

### Microphytes

A total of 98 microphyte plankton taxa were identified in Drwęsa pond in 2011. This number is well within the range considered characteristic for standing water bodies in the temperature zone (TRIFANOVA 1998). However, at individual water sampling dates the species richness of microphytes was small, since the number of taxa ranged from 10 to 28. Even in the period from July to September the number of species was limited, ranging from 14 to 25, as a rule being lower than in twelve ponds of the Wielkopolski National Park (WPN) (MESSYASZ 2001). These ponds were analysed in the summer of 1996 (July – September). At that time the number of taxa ranged from 13 to 75 taxa. In the same time of the year phytoplankton of the pond in Drwęsa had as a rule a lower species richness than ponds in the Wielkopolski National Park.

In phycoflora of the examined pond almost 35% taxa were of indicator value. Most of them are indicators for eutrophic waters, e.g. green algae from the genera *Scenedesmus*, *Ankistrodesmus*, *Tetrastrum*, or *Aphanocapsa* and euglenophytes from the genera *Phacus* and *Trachelomonas* (HÖRNSTRÖM 1981, ROSÉN 1981). Some of them formed relatively numerous populations, thus resulting in an increase in the trophic state level. In turn, cryptophytes were lower in abundance, but they were characterized by a high taxonomic diversity and a lack of indicator species. Thus they had no effect on the assessment of trophic status (REYNOLDS 1984). It needs to be stressed that it is a dynamically changing group in the examined pond, since the results recorded in previous studies in 2009 and 2010 indicated their lower species richness and abundance (BAZALUK 2011).

Diatoms, despite their relatively high species number, accounted for as little as 13% of the microphytes abundance in the analysed pond. It was over two-fold less than e.g. in reservoirs on the Cybinka river (KOWALCZEWSKA-MADURA et al. 2009), or even many times less than in certain ponds in the WPN investigated by MESSYASZ (2001). Diatoms are considered to be an important group of algae in the assessment of water quality. Moreover, they are a particularly preferred group, since they are usually abundant in different types of waters throughout the year (VAN DAM et al. 1994). However, in the analysed pond their representatives were not found among the two most numerous microphytes taxa. They were not observed in the coolest season of the year (January, February). In spring or autumn, as in contrast to other water bodies they formed no abundant populations.

Cyanobacteria are a group of organisms, often creating problems in small water bodies, if appear in great abundance. It is so because they often secrete

substances which deteriorate water quality, while they may also prove toxic to other aquatic organisms and water users (MAZUR-MARZEC et al. 2008). However, in the pond in Drwęsa this group was not very diverse in terms of species composition and its representatives, similarly as diatoms, formed scarce populations. The most abundant representative, i.e. *Aphanocapsa incerta*, being an indicator species for eutrophic waters, appears sporadically, only in the warm season and in not high density. As it was reported by STARMACH (1966), it may be found in the plankton of lakes and ponds, particularly in summer, while it occasionally appears on a mass scale forming the so-called blooms. Also the presence of another cyanobacteria, *Oscillatoria lacustris*, indicated a eutrophic character of pond waters (HÖRNSTRÖM 1981, ROSEN 1981).

### Physico-chemical properties of water

In the analysed pond water temperature did not play any significant role in the regulation of microphytes biomass or their abundance, as indicated by a lack of correlation between water temperature and parameters characterising microphytes, i.e. its abundance and the concentration of chlorophyll *a*. Also other studies showed a slight effect of water temperature on natural algal communities, with a diverse species composition (NYHOLM 1978). This may result from the fact that optimal temperatures are found there throughout the year, since with its increase cold water species are gradually replaced by warm water species. However, generally a positive correlation is expected between water temperature and algal biomass, since the rate of photosynthetic activity and the accompanying algal growth are dependent, among others, on temperature (SZELAĞ-WASIELEWSKA 1992). In turn, a significant, positive correlation was observed between the number of taxa and water temperature – in water at a temperature of approx. 20°C the number of taxa was highest, as high as 28. Shading of the pond in Drwęsa by tree resulted in a lower mean water temperature from April to October (14.6°) than for other mid-field ponds in the Stare Czarnowo District (the Zachodniopomorskie province) (BYSIEWICZ et al. 2012).

A slightly alkaline water reaction recorded in the pond in Drwęsa is highly frequently observed in water bodies located in the Polish Lowland (KAWECKA and ELORANTA 1994). A similar water reaction was found in small water bodies near Poznań on the Cybinka river (e.g. ponds Uli, Ósemka, Baba, Cyganek) (KOWALCZEWSKA-MADURA et al. 2009) as well as water bodies in the Wrocław Plain (ORZEPOWSKI et al. 2008). Also electrolytic conductivity in the analysed pond fell within the range of values frequently reported for water bodies in

western Poland. Similar ranges of values were recorded in a small water body in the Wrocław Plain and in small water bodies in the WPN (ORZEPOWSKI et al. 2008). In spring in the pond in Drwęsa both electrolytic conductivity and the abundance of microphytes were higher than in other seasons. This resulted in a strong, positive correlation between these factors. The negative correlation between the seston dry mass and trophic index ( $I_c$ ) ( $r = -0.640$ ) was caused by the appearance large size species of microphytes, especially those of the *Gymnodinium* genus, which have low trophic index ( $I_c = 1.0$ ). In turn, seston and the concentration of chlorophyll *a* were negatively correlated with electrolytic conductivity, which most probably resulted from the high abundance of small size microphytes, at a relatively high electrolytic conductivity.

Literature sources generally report a directly proportional dependence between the concentration of chlorophyll *a* and seston content (SOLSKI 1962, JONES 1976). Similarly, in the pond in Drwęsa a significant positive correlation was found between these parameters. Seston content well differentiates water quality throughout the year and the pond in Drwęsa may be classified, based on the criteria of KUDELSKA et al. (1981), to second class of water quality. The consistence of the course of annual changes in the levels of seston and chlorophyll *a* makes it possible to forecast amounts of seston based on the concentration of chlorophyll *a*. According to the ranges of values for chlorophyll *a* in the OECD trophic lake classification (LIETH and WHITTAKER 1975, STRAŠKRABA et al. 1979), its high value in the analyzed pond indicates the eutrophic character of these waters. Moreover, based on the Trophic State Index of chlorophyll *a* this water body needs to be considered eutrophic. In turn, taking into consideration the value of the trophic index, calculated on the basis of the abundance of microphytes (mean  $I_c = 1.9$ ) the pond in Drwęsa needs to be considered as mesotrophic water body. The higher trophic level based on the concentration of chlorophyll *a* might result from the presence of numerous ciliates containing symbiotic algae in their cells (FINLAY et al. 1996). Unfortunately, there are no published results about the ciliates in pond in Drwęsa although during microscopic analyses of water samples they were observed in large numbers.

## Conclusions

In the pond in Drwęsa the greatest richness of species was observed among green algae and diatoms. However, chrysophytes, green algae and dinoflagellates were found in greatest abundance. The highest total abundance of microphytes was recorded in spring, while in the second half of the year it was several times lower. Limited abundance were recorded for diatoms, which

results in a limited applicability of this group considered to be a good indicator, for the assessment of trophic state of the investigated pond. Approximately 35% phycoflora of the pond comprised indicator taxa, of which as many as 56% belong to indicators of eutrophy. Water temperature, reaction and electrolytic conductivity were consistent with the ranges reported for many small water bodies. The application of different indicators in the assessment of trophic status produces inconsistent results. Microphytes structure indicated a lower water trophic status, i.e. mesotrophy, while the concentration of chlorophyll *a* and seston content indicated eutrophy. Thus the assessment would have to be extended to include microphytes biomass determined by microscopic examination, since microphyte biomass established on the basis of chlorophyll *a* concentration may be elevated by plankton ciliates containing symbiotic algae.

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**CONTENT OF MACRONUTRIENTS (Ca, Mg, K, P, Na)  
IN INITIAL SOILS AT THE EARLY STAGES  
OF RECLAMATION OF A LIMESTONE MINE\***

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**Key words:** limestone mining, reclamation, macronutrients, initial soils.

**A b s t r a c t**

The paper evaluates the productivity status in initial soils in reclaimed areas after open-cast limestone mining. Two research plots were designated in the post-exploitation excavation, 1 year and 5 years post reclamation, respectively. The initial soil productivity was assessed based on the content of macronutrients such as P, Mg, Ca, K and Na in the surface soil layer, which was 20 cm deep, and determined by methods commonly used in soil science. Based on the contents of macronutrients in the initial soils, low abundance of macroelements were observed in the soils under pine trees, this may be connected with geochemical properties of the mineral material and the pedogenic process that accelerates weathering in carbonate rocks. A moderate enrichment in macronutrients was observed over the years in the top layers of the reclaimed excavation, particularly in potassium, calcium and magnesium.

**ZAWARTOŚĆ MAKROELEMENTÓW (Ca, Mg, K, P, Na) W INICJALNYCH GLEBACH  
NA WYROBISKU PO GÓRNICTWIE WAPIENI W POCZĄTKOWEJ FAZIE  
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### Abstrakt

W pracy przedstawiono ocenę stanu produktywności gleb inicjalnych na zrehabilitowanych terenach po górnictwie odkrywkowym wapieni. Wyznaczono dwie powierzchnie badawcze na wyrobisku poeksploatacyjnym odpowiednio zrehabilitowane po 1 roku i 5 latach. Stan produktywności tworzących się gleb oceniono na podstawie zawartości makroelementów, tj. P, Mg, Ca, K i Na w warstwie gruntu do 20 cm, oznaczonych według powszechnie stosowanych metod w gleboznawstwie. Na podstawie przeprowadzonych badań zauważa się niską zasobność badanych gleb w wymienione makropierwiastki pod nasadzeniami sosny zwyczajnej. Jest to związane z właściwościami geochemicznymi mineralnego gruntu oraz kierunkiem procesu pedogenicznego wpływającego na wietrzenie skał węglanowych. Z upływem kolejnych lat od przeprowadzenia procesu rekultywacji biologicznej zauważa się umiarkowane wzbogacanie wierzchnich warstw w składniki pokarmowe, szczególnie potas, wapń i magnez.

## Introduction

The most noticeable changes in the natural environment caused by open-cast extraction of carbonate raw materials include transformation of the land surface including large areas of limestone mining. The adverse effect on soil is particularly noticeable on the areas degraded by mining excavations (MIKLA-SZEWSKI 1972, SZLAGOWSKI 1993, BAILEY and GUNN 1993, DUBEL 2005, KUSZA 2006, JORDÁN et al. 2009, KUSZA and GOŁUCHOWSKA 2009). It is estimated that lithogenic soil with very low productivity will be formed in the pits where the external layer of overburden was removed and the solid rock was exposed. Productivity of degraded soils is defined as a set of physico-chemical and biological properties of the surface layer soil allowing for the satisfactory development of vegetation (KOWALKOWSKI and RUBINOWSKI 1991, SZLAGOWSKI 1993). The initial soils formed represented a mosaic of soils with different profiles, consisting of the exposed bedrock and weathered surface contaminated with chippings of stones, dust from drilling blast holes and mechanical processing and detonation gases (SZLAGOWSKI 1993). Restoration of soils on the new surface is possible with the appropriate reclamation of post-exploitation excavations. The main effect which is supposed to be achieved through reclamation of such areas is to restore the original state or to achieve a new quality in these areas, which provides opportunities for cost-effective and socially useful activities of agricultural, forestry or other character, while eliminating the burden on the environment (DULEWSKI and WTOREK 2000, BENDER and GILEWSKA 2004, STRZYSZCZ 2004, FABIAŃSKA 2005, KUSZA 2007, JORDÁN et al. 2009, KASZTALEWICZ and PTAK 2011).

The process of reclamation of soilless mining areas, with all the appropriate technical and biological procedures, is utilized to form some properties of the initial soils which have a significant effect on the success of the whole project. The essential importance is attached to susceptibility of the rock material to



weathering processes and properties of the formations found in the upper layers of the soil (KRZAKLEWSKI and MIKLASZEWSKI 1996, KOWALIK and WÓJCIK 2005, WÓJCIK and KOWALIK 2006, KUSZA and GOŁUCHOWSKA 2009). The new rock material formed in the subsequent geological period is soft and prone to weathering processes that foster reclamation and helps quickly achieve the desired effect (KUSZA and GOŁUCHOWSKA 2009). Formation of morphological profile and all the basic characteristics of the soil created is also affected by the abundance of nutrients which are easily available to plants (WÓJCIK and KOWALIK 2006, DOMSKA and RACZKOWSKI 2008). For this reason, numerous researchers have considered the quantity and quality of initial nutrients accumulated in initial soils as one of the main criteria for assessment of the effectiveness of reclamation and a determinant of soil productivity in mining areas.

The focus of the present study was on the content of such nutrients as carbon, nitrogen, phosphorus, potassium, magnesium, calcium and sodium in the area of a post-exploitation excavation in an open-cast limestone mine in GóraŹdŹe.

## **Materials and Methods**

The GóraŹdŹe deposit of Triassic limestone is located in Opole Silesia, Poland. Characteristic in the GóraŹdŹe Mine is the presence of thinner or thicker layers of sand and gravel coming from postglacial period, related to small changes of level of the terrain. Depth of the excavation reaches about 35 metres in relation to surface of the terrain (KUSZA and GOŁUCHOWSKA 2009). The surface of the GóraŹdŹe deposit is covered with overburden as Quaternary sands and clays and degraded rubble with thickness ranging from 0.3 m to 13.0 m (DRESZER 2003). Mineral waste builds the overburden regular used to reclamation of post-exploitation excavations. Due to the heterogeneity of deposition of these materials, the surface of excavation is characterized by large mosaic of granulation mineral material. The surface of excavation mainly builds sandy formations reached to solid rock horizon, it is to 30 cm and a strongly skeletal loam formations that remain particularly in upper horizons (humic initial horizons).

The mine is mostly surrounded by woodlands and overgrown by coniferous trees. As a result, the horizon radius is small so landscape and view values are in significant. Reclaimed land on which mining has been completed, brings diversity to relief, such as numerous small ponds and depressions of the terrain. Both the introduced stand and the stand from the succession form a specific biocoenosis and positively influence soil – formative processes. Current management strategies for post-mining areas in the GóraŹdŹe Mine are oriented towards forest reclamation (KUSZA and GOŁUCHOWSKA 2009).

Two research plots with dimensions of 12 x 3.3 m were designated at the bottom in of the excavation in the Górażdże Limestone Mine, after 1 or 5 years of reclamation, respectively. The study included just young reclaimed surfaces objective to showing changes in the properties of soils formed in the first years of cultivation, deciding for adroitness introduced seedlings of trees. The designated areas are located at the bottom of the excavation, just a hundreds meters from each other, in different habitat conditions. One-year plot is located in close proximity to the quarry wall and technological road; overgrown by a single grassy vegetation. Five-year plot located in close proximity to the pond and drainage ditch; heavily overgrown by scrub and non-forest vegetation. Before seeding the plants, the authors used fertilizers in the two plots, containing N, P, K with the following composition: nitrogen ( $80 \text{ kg ha}^{-1}$ ) in the form of 34% ammonium nitrate ( $90\% \text{ N}$ ,  $210 \text{ kg ha}^{-1}$ ) and 20% ammonium sulfate ( $10\% \text{ N}$ ,  $40 \text{ kg ha}^{-1}$ ), phosphorus ( $100 \text{ P}_2\text{O}_5 \text{ kg ha}^{-1}$ ) in the form of 46% of granulated superphosphate ( $220 \text{ kg ha}^{-1}$ ) and potassium ( $120 \text{ kg K}_2\text{O ha}^{-1}$ ) in the form of 56% of potassium salt ( $215 \text{ kg ha}^{-1}$ ). The fertilization is used after technology reclamation. The experimental plots are now covered with pine trees (*Pinus sylvestris* L.) introduced in the planting density  $0,6 \times 1,2$  meters as a form of biological reclamation. Nine samples were collected randomly from the surface layer (20 cm) of two plots in the spring of 2011 to represent the whole surface. The physical and chemical analyses, which are commonly used in soil science, were carried out for the soil samples (OSTROWSKA et al. 1991) to determine the status of initial productivity of soils in the areas studied.

In soil samples were used to determine:

- granulometric composition: grain size distribution (soil texture) Casagrande aerometric method modified by Proszynski, fractions of sand sieve method according to *Jakość gleby...* PN-ISO 11277:2005;
- oil pH: potentiometry method according to *Jakość gleby...* PN-ISO 10390:1997; conductivity: conductometric method according to *Jakość gleby...* PN-ISO 11 265 + ACI: 1997; calcium carbonate content: Scheibler volumetric method according to *Jakość gleby...* PN-ISO 10693:2007; organic carbon content: Tiurin modified method according to *Jakość gleby...* PN-ISO 14235:2003; total nitrogen content: Kjeldahl method according to *Jakość gleby...* PN-ISO 11261:2002;
- content of available phosphorus according to *Analiza chemiczno-rolnicza...* PN-R-04023: 1996;
- content of potassium, magnesium, calcium, sodium: method of emission spectrometry with inductively coupled plasma (ICP-AES) after prior mineralization in acid mixture of HCl and  $\text{HNO}_3$  in a ratio of 3:1.

The results obtained from the measurement of macroelement contents were analyzed with Tukey's test, with significance level set at  $p = 0.05$ .

## Results and Discussion

### Granulometric composition

Granulometric composition of the formations that represented the overburden layer in the pit for future biological reclamation procedures is an important factor. This significantly affects grain size distribution in the soils formed in the post-exploitation areas of the limestone mine as well as their chemical and physicochemical properties. The specificity of soil conditions observed in post-exploitation excavation depends on the quality of mineral tracks deposited in them (KUSZA 2007, KUSZA and GOŁUCHOWSKA 2009). Heterogeneity of mineral materials and the lack of selectively removed overburden mean that excavations are characterized by a large mosaic of grains in the material deposited in the pits (KUSZA and GOŁUCHOWSKA 2009). Granulometric composition of the 20 cm layer in the research plots designated in the excavation in the Góraźdże Mine was characterized by a dominant share of sandy formations, according to the classification of soil texture (PTG 2009), belonging to a subgroup of sand (S) in both studied plots (Table 1).

It should be noted that the soils formed from sandy materials contain minimum amounts of nutrients and are highly permeable, which consequently leads to only a small amount of water available to plants (ZAWADZKI 1999). SIUTA (1998) and STRZYSZCZ (2004) defined sands as a very defective soil-forming material, where only forest vegetation is able to form the initial soil and protect it from degradation.

### Reaction

Soil reaction (pH) in the soils which were formed in the study represents, apart from grain composition, another important precondition for availability of nutrients which affects soil-forming processes (STACHOWSKI 2005, BENDER et al. 2005, KUSZA 2007, KACPRZAK and BRUCHAL 2012). Soils composed of sand materials are more vulnerable to changes in pH, which is associated with high permeability of grounds, lack of humus and poor sorption complex (KUSZA and GOŁUCHOWSKA 2009). Soil reaction in grounds forming the surface layer in the research plots was at the alkaline level: 7.8 for the 1-year plot and 7.6 for the 5-year plot (Table 2).

Table 1

## Granulometric composition of analyzed soils

Grain size fraction	Percentage share of fraction [by % weight]
Surface (age in years): 1 Deph of sampling: 0–20	
> 2	5
< 2	95
Fraction of sand	
2–1	10
1–0.5	34
0.5–0.25	23
0.25–0.1	27
0.1–0.05	1
$\Sigma > 0.005$	95
Fraction of silt	
0.05–0.02	2
0.02–0.005	1
0.005–0.002	0
$\Sigma > 0.002$	3
Fraction of clay	
> 0.002	2
Group and subgroup	pl
Surface (age in years): 5 Deph of sampling: 0–20	
> 2	92
< 2	8
Fraction of sand	
2–1	12
1–0.5	36
0.5–0.25	23
0.25–0.1	18
0.1–0.05	4
$\Sigma > 0.005$	93
Fraction of silt	
0.05–0.02	2
0.02–0.005	2
0.005–0.002	0
$\Sigma > 0.002$	4
Fraction of clay	
> 0,002	3
Group and subgroup	ps

pl, ps – sand (S)

Table 2  
Chosen physicochemical properties of soils reclaimed into forest in the pit of limestone mine

Specification	pH	Conductivity [ $\mu\text{s cm}^{-1}$ ]	Organic C [%]	Total N [%]	Calcium carbonate [%]
Deph: 0–20 cm	1-year plot				
Mean	7.8	55.9	0.2	0.028	7.5
Range	7.6–7.8	42.7–77.8	0.1–0.3	0.027–0.028	6.3–10.2
Standard deviation	0.1	9.2	0.1	0.001	1.1
Deph: 0–20 cm	5-year plot				
Mean	7.6	82.9	0.6	0.06	6.5
Range	7.4–7.7	55.2–118.8	0.4–0.8	0.03–0.07	4.7–7.9
Standard deviation	0.1	18.3	0.2	0.01	1.2
HSD $p = 0.05$	0.2	26.9	0.4	0.03	0.9

Over the subsequent years of biological reclamation, the soils formed in the post-calcareous excavation showed a small decrease in pH in the surface layer, which is confirmed by the results obtained for pH and studies carried out by KUSZA and GOŁUCHOWSKA (2009) in two dumping grounds in the area of a former limestone mine, forested 45 years ago. The initial anthropogenic soils present in this site exhibited constant alkaline pH at the level of from 7.3 to 7.6 in the Kamień Śląski Mine and 7.3 to 7.9 in Strzelce Opolskie Mine.

### Calcium carbonate

The low acidification rate in surface layers of initial soils in post-mining lands is caused by significant contents of calcium carbonates (SPYCHALSKI and GILWESKA 2008), which reached the levels of 7.5% and 6.5% in the layer with thickness of 20 cm in 1-year plot and 5-year plot, respectively. According to STACHOWSKI (2005), this significant content of calcium carbonate results from genetic properties of the overburden material used for forming the excavation surface.

### Conductivity

Soils formed from overburden material showed low salinity in surface layers, which, in both 1-year and 5-year plot studied, did not exceed  $100 \mu\text{s cm}^{-1}$  (Table 2). Over the subsequent years after biological reclamation, the levels of

soil conductivity in the post-calcareous excavations remained at an average of 100–280  $\mu\text{s cm}^{-1}$  in a study carried out by KUSZA (2007) in Opole Limestone Mines and 275  $\mu\text{s cm}^{-1}$  in a mine in Spain examined by JORDAN et al. (2009). A decrease in conductivity of surface levels of soil on older fields should be associated with an increased content of potassium, magnesium and nitrogen (KUSZA and GOŁUCHOWSKA 2009).

### **Organic matter**

Enrichment of subsurface layers with organic matter over the years of forestation is an important factor in the activation of soil forming processes in post-mining areas (GILEWSKA and OTREMBKA 2004, WÓJCIK and KOWALIK 2006). The source of organic matter in the post-mining areas are dead parts of higher plants, accompanying vegetation and underground parts of plants. The studied areas of forestation (1-year and 5-year plants) show significant changes in the dynamics of organic matter content in the surface layer. In the 5-year surface, the contents of nitrogen and carbon in the layer with thickness of 20 cm amounts to 0.06% of total nitrogen, 0.6% of organic carbon and was twice higher compared to the 1-year surface (Table 2). The noticeable increase in the content of organic matter in the subsequent years of forestation in reclaimed areas was also demonstrated in a study by KUSZA (2007). The dumping grounds in the two limestone mines examined by the author and results of the studies showed that the content of organic matter in the surface layer was at a level of 0.72% of organic carbon and 0.018% of total nitrogen in Kamień Śląski Mine and 0.61% organic carbon and 0.020% total nitrogen in Strzelce Opolskie Mine. However, these levels of accumulated organic matter were small considering that these areas were forested 45 years before. The results obtained by the author showed that the post-mining soil remained over the years the ecosystem which accumulated and immobilized nitrogen (BENDER and GILEWSKA 2004, SPYCHALSKI and GILEWSKA 2008).

### **Macroelements**

#### **Phosphorus**

One effect of changes that occurred in the post-exploitation excavation, besides the accumulation of organic matter and weathering processes in the overburden material, was an increase in abundance of plant nutrients present in the surface layers, especially in phosphorus (WÓJCIK and KOWALIK 2006).

A moderate increase in abundance of available phosphorus in the 20 cm surface layer of the soils that was formed was observed over the years in the research plots designated for the study. This was caused by the progressive accumulation of organic matter and the change in pH that promotes assimilation of the nutrients. The content of phosphorus in the surface layer in the analyzed plots (1-year and 5-year) was low: 1.0 and 1.2 mg  $P_2O_5$  per 100 g soil (Table 3) and this difference in the content of  $P_2O_5$  was not statistically significant. The low content of available phosphorus in the initial phase of reclamation of post-calcareous excavations was also demonstrated in studies by KUSZA (2006) (Tarnów Opolski excavation, Poland) and JORDÁN et al. (2009) (Alicante excavation, Spain). The values of available phosphorus in the layer of up to 20 cm measured by these authors exhibited at content of 1.5 mg  $P_2O_5$  per 100 g soil (Tarnów Opolski) and from 1.5 to 1.9 mg  $P_2O_5$  per kg soil (Alicante). For a small phosphorus content may correspond to the higher content of calcium and magnesium, because in an alkaline environment the phosphate ions react with calcium and magnesium compounds in the form of sparingly soluble calcium and magnesium phosphates. Losses of phosphorus in surface levels could be due to washing out or uptake of this element by the plants (ZAWADZKI 1999). In general, post-mining grounds are poor in phosphorus available to plants (GILEWSKA and SPYCHALSKI 2004), hence the need for their fertilization in the form of compost obtained from sewage sludge or increased doses of mineral fertilizers (KUSZA and GOŁUCHOWSKA 2009).

Table 3  
Content of macronutrients in soils reclaimed into forest in the pit of limestone mine

Specification	$P_2O_5$ [mg 100 g <sup>-1</sup> soil]	K [mg 100 g <sup>-1</sup> soil]	Ca [mg 100 g <sup>-1</sup> soil]	Mg [mg 100 g <sup>-1</sup> soil]	Na [mg 100 g <sup>-1</sup> soil]
Deph: 0–20 cm	1-year plot				
Mean	1.0	9.1	42.5	6.0	1.7
Range	0.9–1.1	9.0–9.2	40.2–45.5	5.5–6.2	1.4–1.9
Standard deviation	0.1	0.1	1.8	0.2	0.2
Deph: 0–20 cm	5-year plot				
Mean	1.2	11.5	48.8	8.5	1.5
Range	1.0–1.4	8.8–15.9	44.1–51.6	7.8–9.1	1.2–1.8
Standard deviation	0.2	2.3	2.6	0.5	0.2
HSD $p = 0.05$	0.2	2.4	6.3	2.5	0.1

### **Potassium**

Potassium belongs to macronutrients which significantly affect growth and development of root systems in plants, especially during the period of adaptation in the first years after planting (KUSZA and GOŁUCHOWSKA 2009). In contrast to phosphorus, the content of potassium in the layer studied increased over the years from the biological reclamation, at a level of 9.1 mg per 100 g soil in the 1-year plot and 11.5 mg per 100 g soil in the 5-year plot (Table 3). Higher contents of potassium in the 20 cm layer compared to the content of phosphorus were also found by JORDAN et al. (2009) in the Alicante excavation, where the content of potassium ranged from 24 to 79 mg per kg soil. In the case of high concentrations of calcium ions, as it was on the analyzed plots, uptake potassium may be limited (ZAWADZKI 1999). This slowly enrichment of the surface layer in potassium in the post-calcareous areas can be attributed to decomposition of organic matter supplied to the post-exploitation excavation with mineral overburden obtained from more fertile forest areas (KUSZA and GOŁUCHOWSKA 2009).

### **Calcium**

Of all the macroelements, the greatest abundance in the designated research plots was found for calcium. The contents of this element in the both 1-year and 5-year plots were by several times greater than the values measured for other elements (Table 3). This is connected with a high content of this element in the solid rock, especially their abundance in  $\text{CaCO}_3$ . The presence of calcium in the initial soils that were formed in the post-exploitation excavation have a neutralizing effect on acidic products of decomposition of organic matter and thus on the slow decrease in soil reaction in these kinds of grounds (GAŚIOREK and NICIA 2010). The results obtained during the measurements of calcium content in the grounds after limestone mining are supported study by JORDÁN et al. (2009) carried out in one of the limestone mines in Spain, where the content of calcium in the 20 cm layer was the highest among the macronutrients studied and reached the values ranging from 2.1 to 3.1 g per kg soil.

### **Magnesium**

Magnesium is another macronutrient which significantly affects the productivity of post-mining soils. Combined with calcium, this element has a buffering effect on decomposition of organic matter and prevents rapid



changes in pH in the soils formed in the post-calcareous areas (GAŚIOREK and NICIA 2010). The content of magnesium in the research plots in the initial phase of reclamation remained at low level (Table 3), which was also reported by the findings obtained by KUSZA (2006) in the post-exploitation pit of the Tarnów Opolski Limestone Mine, ranging from 8.9 to 10.5 mg per 100 g soil in the 20 cm layer. However, one demonstrated positive effect was the progressive increase in the content of magnesium by 2.5 units observed during the years that have passed since the biological reclamation. This slowly enrichment of the mineral surface layers in plant nutrients, especially magnesium, is undoubtedly the result of the accumulation of the metabolites of organic matter and weathering processes in the mineral soil material (WÓJCIK and KOWALIK 2006). Despite concentration of magnesium in the soil, it may not necessarily be fully absorbed by the plants, because on the uptake of magnesium affected by a high concentration of Ca ions in the soil solution (ZAWADZKI 1999).

### **Sodium**

The content of sodium in the mining areas is related to salinity. Grounds after limestone mining are generally characterized by a low concentration of inorganic salts present in the soil, this was confirmed by the low values of conductivity in the studied plots—55,9  $\mu\text{S cm}^{-1}$  1 year plot and 82,9  $\mu\text{S cm}^{-1}$  5 year plot. Similar the values measured in soils from post-calcareous excavations reclaimed 45 years before, reported in a study carried out by KUSZA (2007), ranging from 150 to 280  $\mu\text{S cm}^{-1}$ . The surface layers of the soil formed in the post-exploitation excavation of the GóraŹdŹe Mine exhibited very low contents of sodium, maintained at at similar level in the analyzed surface layers in both 1-year and 5-year plots (Table 3), despite the years that have passed since reclamation planting. The contents of sodium obtained in the study of the soils that were formed in the mining pit suggest a low concentration of easily soluble salts in the soils after open-cast limestone mining, especially in the initial phase of their reclamation.

### **Conclusions**

The results obtained from the present study, carried out on a excavation of a limestone mine in Poland, focused on the content of basic macronutrients after 1 year and 5 years following reclamation planting, lead to the following conclusions:

1. Physical and chemical analyzes carried out in the layer of soil with the thickness of 20 cm confirm a beneficial direction of soil forming processes stimulated by biological reclamation.

2. Initial soils in the experimental plots designated in the post-exploitation excavation were characterized by low contents of potassium, phosphorus and sodium in the 20 cm layer: largely depend on geochemical properties of soil and the pedogenic process that promotes weathering of carbonate rocks.

3. Among all the elements studied, the highest contents was found for calcium and magnesium related to limestone properties. The presence of these cations in the studied layers of soil caused an increase in the pH of the surface layers.

4. The content of macronutrients (Ca, Mg, K, P, Na) in the surface layer of the research plots increased moderation with time suggesting the slowly susceptibility of rock to weathering processes that allow for achievement of a satisfactory level of soil productivity.

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**CONFIRMATION OF HYBRIDISATION BETWEEN  
RIVER LAMPREY *L. FLUVIATILIS*  
AND BROOK LAMPREY *L. PLANERI* FROM *IN SITU*  
EXPERIMENTS**

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**Key words:** *Petromyzontidae*, paired species, ammocoete, reciprocal cross, speciation.

**Abstract**

Interspecific mating and taxonomic status of river lampreys *Lampetra fluviatilis* and brook lampreys *Lampetra planeri* has been repeatedly discussed in recent years. Molecular studies fail to deliver clear separation between two species and the number of reported communal spawning cases across the natural range is constantly increasing. Here, we present results of interspecific breeding experiment *in situ* involving both species of *Lampetra* genus in Lithuanian lowland rivers. For the experiment, we collected the spawning individuals in communal redds and manually striped them into modified salmonid incubation boxes to incubate lamprey eggs in the same redds under ambient conditions. We have received viable reciprocal crosses of ♂ *L. planeri* × ♀ *L. fluviatilis*, ♂ *L. fluviatilis* × ♀ *L. planeri*, as well as viable ammocoetes in the control group of *L. fluviatilis* × *L. fluviatilis*. The experiment suggests that, in case of communal spawning, the production of viable hybrids is possible. The results as well supports hypothesis of two different ecotypes of a single *Lampetra* species.

**HYBRYDYZACJA MINOGA RZECZNEGO *L. FLUVIATILIS*  
Z MINOGIEM STRUMIENIOWYM *L. PLANERI* POTWIERDZONA DOŚWIADCZENIEM  
*IN SITU***

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**Słowa kluczowe:** *Petromyzontidae*, larwy minogów, krzyżowanie międzygatunkowe, specjacja.

## Abstrakt

Status taksonomiczny i zjawisko hybrydyzacji międzygatunkowej minoga rzecznej *Lampetra fluviatilis* z minogiem strumieniowym *Lampetra planeri* były wielokrotnie dyskutowane w ostatnich latach. Badania molekularne nie dostarczyły wyraźnych dowodów na przynależność tych bezszczękowców do oddzielnych gatunków, co więcej rośnie liczba doniesień o wspólnie odbywanym tarle na terenie całego zasięgu naturalnego występowania tych ryb. W pracy prezentujemy wyniki eksperymentu krzyżowania międzygatunkowego *in situ* z udziałem obu gatunków z rodzaju *Lampetra* z litewskich rzek nizinnych. Do eksperymentu użyto tarlaków złowionych podczas tarła. Ikrę umieszczono w zmodyfikowanym aparacie wylęgarniczym, zapładniano i umieszczano w okolicy już zbudowanych gniazd. Uzyskano krzyżówki *L. planeri* × *L. fluviatilis* oraz *L. fluviatilis* × *L. planeri*. Wykazano, że w przypadku wspólnego tarła minogów rzecznych i strumieniowych mogą powstawać żywotne mieszańce obu gatunków. Wyniki mogą też potwierdzać hipotezę o dwóch różnych ekotypach jednego gatunku w obrębie rodzaju *Lampetra*.

## Introduction

According to HARDISTY and POTTER (1971), the lack of observation of successful interspecific mating of brook lampreys *Lampetra planeri* (Bloch, 1784) and river lampreys *Lampetra fluviatilis* L. in the field supports the theory of two distinct species. However, such communal spawning has been observed in a number of rivers across Europe (HUGGINS and THOMPSON 1970, LASNE 2010). Additionally, post-zygotic viability of *L. fluviatilis*, *L. fluviatilis* landlocked individuals and *L. planeri* has been shown via *in vitro* experiment by HUME et al. (2013a). This phenomenon of communal spawning is also frequently observed in rivers of eastern and western Lithuania. According to LASNE (2010) interspecific communal spawning in the same redds is the first prerequisite for hybridisation to occur between *L. fluviatilis* and *L. planeri*. Therefore, we carried out an experimental *in situ* hybridisation between *L. fluviatilis* and *L. planeri* individuals collected in communal redds to test whether crossbreeding is possible under natural conditions.

## Materials and Methods

The experiment was carried out in three rivers in the North West Lithuania: Šventoji, Blendžiava, Salantas. As *L. fluviatilis* and *L. planeri* were already described by ZHUKOV (1965) to occur sympatrically in the upper tributaries of the rivers Nemunas and Vistula, two study sites were selected in the Nemunas river basin. One site was situated in the Salantas River 1<sup>st</sup> order tributary and one in the Blendžiava River 2<sup>nd</sup> order tributary of the Minija River which is one of major tributaries of river Nemunas. The third site was selected in the Šventoji River which is a cross border river of Lithuanian and Latvia, a direct tributary of the Baltic Sea, sustaining abundant spawning populations of *L. fluviatilis*.

*L. fluviatilis* and *L. planeri* individuals were captured in three communal redds (one redd per study site) in early May 2012, during the peak of spawning period. Majority of lamprey spawners were individually collected by dip net as this was the least stressful method to avoid any undesirable effect on egg fertility. Lampreys that displayed avoidance reaction were trapped in fyke nets installed 1–1.2 m downstream of the redd tailspills. In order to eliminate any possibility of egg fertilization by undetected *L. planeri* males, we thoroughly checked substrate in the redds and redd tailspills. *L. planeri* individuals are known to be found burrowed in red substrate (personal observation).

All lampreys were collected while spawning in redds. Females and males of both species possessed distinctive sexual features typical for mature individuals: females – post-cloacal fin fold, males – genital papilla extending from the cloaca (RENAUD 2011). All crosses were performed between unique pairs of lampreys. As spent females were also present in redds, females were selected according to body fill and visible eggs through translucent skin near the cloaca. Males for crossbreeding were selected randomly. *L. planeri* and *L. fluviatilis* spawners were anaesthetised by 2-phenoxy ethanol 0.5 ml l<sup>-1</sup>. Gametes of both sexes were hand stripped into modified salmonid incubation boxes (RUBIN 1995, NIKA 2011). Incubation boxes prior to realise of gametes were placed in 0.75 l beaker filled with stream water taken above redds. Hybridisation was tested among reciprocal crosses of *L. planeri* × *L. fluviatilis* and control group crosses of *L. fluviatilis* × *L. fluviatilis*. Each cross group was placed in individual incubation box. Incubation boxes were made of a PVC cylinder (diameter: 8 cm; height: 10 cm; volume: 500 cm<sup>3</sup>). The box was sealed with double layer of mesh: outer flexible protective PVC mesh (mesh size 6 x 6 mm) and inner mesh (mesh size 200 µm) to secure egg compartments from fine sediment and to prevent hatched larvae from escaping (Figure 1a). PVC hoops were tightened with clamps on the top and bottom parts of the box to support its structure.

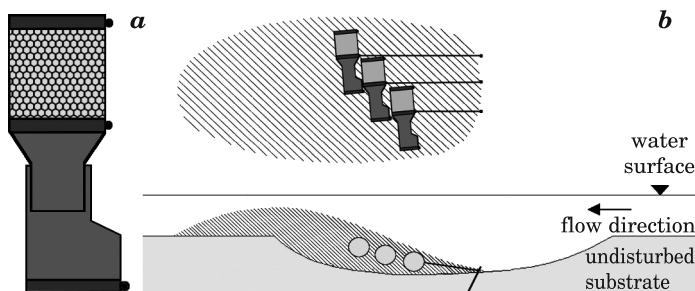


Fig. 1. Experimental egg incubation box (a) and scheme of incubation box placement in redd tailspills (b)

We applied minimal force to strip out only ovulated eggs into incubation boxes as ovulation in lampreys is known to start at the posterior end of the ovary (LARSEN 1970). Two thirds of an ovulated egg is covered with a thick layer of sticky jelly (LARSEN 1970); this enables instant fixation of eggs to the surface of the inner mesh leaving the animal pole exposed for spermatozoa to enter. The number of retrieved eggs varied considerably from a few dozen of *L. planeri* to several thousands of *L. fluviatilis*. According to KILLE (1960), lamprey spermatozoa can survive in fresh water for about 50 seconds. Considering this fact boxes were kept in the water reservoir and hand stirred for a minute.

The boxes with fertilized eggs were positioned at the front of the redd tailspill or crest and anchored to the bottom to minimize losses (Fig. 1b). Despite our preventive measures, all three incubation boxes were lost in the Salantas River and one incubation box in the Sventoji River, either due to the current or retrieved by local anglers. Thus, the results are presented of only two study sites – Šventoji and Blendžiava Rivers. The remaining boxes were checked every third day for hatched larvae.

## Results and Discussion

In the three communal redds selected for this experiment we caught a total of 46 lampreys. Redds were dominated by river lamprey, the ratio of brook to river lampreys in redds varied between 0.35–0.45. Both male and females of *L. planeri* and *L. fluviatilis* were present in redds. The males to females ratio of both species in the study sites was close to equal, in *L. fluviatilis* it varied between 0.45–0.55 and in *L. planeri* between 0.25–0.71. Overall 188 larvae hatched during the study period of which 19 were hybrids either  $\sigma^{\text{♂}} L. planeri \times \text{♀ } L. fluviatilis$  or  $\sigma^{\text{♂}} L. fluviatilis \times \text{♀ } L. planeri$  and 169 *L. fluviatilis* larvae which were used as a control group (Table 1). The numbers of hatched larvae in each box ranged due to a different amount of eggs in every box. The experiment was designed to meet conditions closest to natural ones without the use of any egg de-adhesion compounds. Due to highly adhesive properties of eggs we stripped them directly into incubation boxes. Therefore, it was impossible to quantifying the number of eggs and later on estimate hybrid success between cross groups. The emergence of larvae in all incubation boxes was observed at the end of May after 21 days of incubation. We kept larvae in boxes for 41 day after hatching and during this time no mortality of hybrids were observed which indicates no increased mortality of hybrids in initial life stage.



Table 1

Number of lamprey larvae retrieved from incubation boxes

River	Hybridisation directions	Number of incubation boxes	Number of larvae
Salantas	♂ <i>L. planeri</i> × ♀ <i>L. fluviatilis</i> *	1	–
	♂ <i>L. fluviatilis</i> × ♀ <i>L. planeri</i> *	1	–
	♂ <i>L. fluviatilis</i> × ♀ <i>L. fluviatilis</i> *	1	–
Blendžiava	♂ <i>L. planeri</i> × ♀ <i>L. fluviatilis</i>	1	18
	♂ <i>L. fluviatilis</i> × ♀ <i>L. fluviatilis</i>	1	21
Šventoji	♂ <i>L. planeri</i> × ♀ <i>L. fluviatilis</i> *	1	–
	♂ <i>L. fluviatilis</i> × ♀ <i>L. planeri</i>	1	1
	♂ <i>L. fluviatilis</i> × ♀ <i>L. fluviatilis</i>	1	148

\* lost incubation box

Our experiment shows that communal spawning of *L. planeri* and *L. fluviatilis* could produce interspecific hybrids, and they could stay alive for a prolonged period of time. These findings may also be applicable to possible hybridisation among other known paired species of lampreys such as *Ichthyomyzon unicuspis* and *I. fossor* (MORMAN 1979) or *Ichthyomyzon gagei* and *I. castaneus* (COCHRAN et al. 2008). During the collection of data, we observed *L. planeri* individuals burrowed in tailspills of redds what can be seen as one of strategies to fertilize eggs in a redd substrate and an argument in support of sneaky male hypothesis (HUME et al. 2013b). However, we observed both sexes of *L. planeri* in substrate of communal redds and consider this to be energy efficient behaviour as sexually mature adults also burrow in fine sand in pre-spawn period (RENAUD 2011). The ammocoetes require prolonged fluvial conditions for growth and later on access to large water bodies. Life history strategy makes it difficult to determine fertility of supposedly anadromous hybrids, thus, limiting a range of methods that can be applied to assess taxonomic status of the two *Lampetra* species.

## Conclusions

We have confirmed that *L. fluviatilis* and *L. planeri* not only spawn in communal redds, but are also potentially capable of producing hybrids under natural condition. This is highly probable in case of simultaneous egg and milt release of the two species. The taxonomic status both species is intensively discussed across the Europe with little direct evidence to support or refuse that these are either one or two species. This can hardly be determined without hybridisation and gene flow studies between a number of sympatric popula-

tions. The Nemunas and Vistula River basins are one of the few regions where the ranges of both species overlap. This area provides a great opportunity to study these two species *in situ* and help determine they are truly separate species or not.

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**COMPARISON OF LAND SUITABILITY  
FOR DIFFERENT IRRIGATION METHODES  
IN SHOIBIEH PLAIN (SW IRAN)**

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**Key words:** drip irrigation, soil series, sprinkler irrigation, surface irrigation, land suitability evaluation.

**Abstract**

The main objective of this research is to evaluate and compare land suitability for different irrigation methods based upon a parametric evaluation system in an area of 49 622 ha in the Shoibieh Plain, SW Iran. The obtained results showed that for 18 804.64 ha (37.9%) of the study area surface irrigation method was highly recommended; whereas for 36 046.49 ha (72.6%) of the study area a sprinkler irrigation method would provide to be extremely efficient and suitable. The results demonstrated that by applying sprinkler irrigation instead of surface and drip irrigation methods, the arability of 48 805.43 ha (98.3%) in the Shoibieh Plain will improve. The comparison of the different types of irrigation revealed that sprinkler irrigation was more effective and efficient than the drip and surface irrigation methods for improving land productivity. It is of note however that the main limiting factor in using sprinkler irrigation methods in this area was calcium carbonate content.

**PORÓWNANIE PRZYDATNOŚCI RÓŻNYCH METOD NAWADNIANIA GRUNTÓW  
W SHOIBIEH PLAIN (PŁD.-ZACH. IRAN)**

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**Słowa kluczowe:** nawadnianie kropelkowe, deszczowanie gleb, nawadnianie powierzchniowe, ocena przydatności gruntów.

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### Abstrakt

Głównym celem badań była ocena i porównanie przydatności różnych metod nawadniania gruntów w oparciu o system oceny parametrycznej na obszarze 49 622 ha w Shoibieh Plain w Iranie. Dowiedziono, że dla 18 804,64 ha (37,9%) badanego obszaru najbardziej zalecana jest metoda nawadniania powierzchniowego, natomiast dla 36 046,49 ha (72,6%) powierzchni korzystna i niezwykle wydajna jest metoda deszczowania. Rezultaty badań wskazują, że po zastosowaniu deszczowania, zamiast metod nawadniania powierzchniowego i kropelkowego, wzrośnie żyzność gleb na powierzchni 48 805,43 ha (98,3%) w Shoibieh Plain. Z porównania różnych rodzajów nawadniania wynika, że deszczowanie skuteczniej i efektywniej wpłynęło na poprawę produktywności gruntów niż kropelkowe i powierzchniowe metody nawadniania. Należy jednak zauważyć, że głównym czynnikiem ograniczającym wykorzystanie metody deszczowania na tym obszarze była zawartość węglanu wapnia.

## Introduction

In Shoibieh Plain located in the Khuzestan Province, in the South West of Iran irrigation practices can be very expensive and may cause negative phenomena, such as salinisation, alkalization, drainage limitation, soil erosion and finally land degradation. Main causes of these limitations are: lack of appropriate irrigation water management, leaching, and lack of appropriate drainage facilities.

On the other hand, due to the depletion of water resources and an increase in population, the extent of irrigated area per capita is declining and irrigated lands now produce 40% of the food supply (HARGREAVES and MEKLEY 1998). According to FAO methodology (1976) land suitability is strongly related to „land qualities” including erosion resistance, water availability and flood hazards which are derived from slope and length, rainfall and soil texture. SYS et al. (1991) suggested a parametric evaluation system for irrigation methods which was primarily based on physical and chemical soil properties.

DENGIZ (2006) also compared different irrigation methods including surface and drip irrigation in the pilot fields of Central Research Institute, Lkizce research farm located in Southern Ankara. He concluded that the drip irrigation method increased the land suitability by 38% compared to the surface irrigation method.

GIZACHEW and NDAO (2008) evaluated the land suitability for surface (gravity) and drip (localized) irrigation in the Enderta District, Tigray, Ethiopia, using Sys's parametric evaluation systems. Drip irrigation can be a good method of irrigation in this region, if it is managed properly (best design, filters, etc.)

BROU and WOLDEGIORGIS (2009) performed a land suitability evaluation for two types of irrigation i.e., surface irrigation and drip irrigation, in the Kilte Awulaelo District – Tigrayregion – Ethiopia using the suggested parametric evaluation. As the study area is composed of heterogeneous physiographic features, dominantly from undulating to steep scarp, the drip irrigation

suitability gave more irrigable areas compared to the surface irrigation practice, due to the topographic (slope), soil (depth and texture), surface stoniness and drainage limitations worked out in the surface irrigation suitability evaluation.

ALBAJI et al. (2009a) compared the suitability of land for surface and drip irrigation methods according to a parametric evaluation system in the plains west of the city of Shush, in the southwest Iran. The results indicated that a larger amount of the land (30,100 ha – 71.8%) can be classified as more suitable for drip irrigation than surface irrigation.

Using irrigation, farmers have to apply a lot of water to the crops and soil. In order to avoid land degradation, irrigation water and techniques must be compatible with the soil properties. For this reason, it is necessary to evaluate the suitability of land for irrigation. Therefore, the main objective of this research is to evaluate and compare land suitability for surface, sprinkler and drip irrigation methods based on the parametric evaluation systems for the Shoibieh Plain, in the Khuzestan Province, South West of Iran.

## Materials and Methods

### Study Area

The present study was conducted in an area about 49 622 hectares in the Shoibieh Plain, in the Khuzestan Province, located in the South West of Iran during 2010–2011 (Figure 1). The study area is located 40 km North of the city

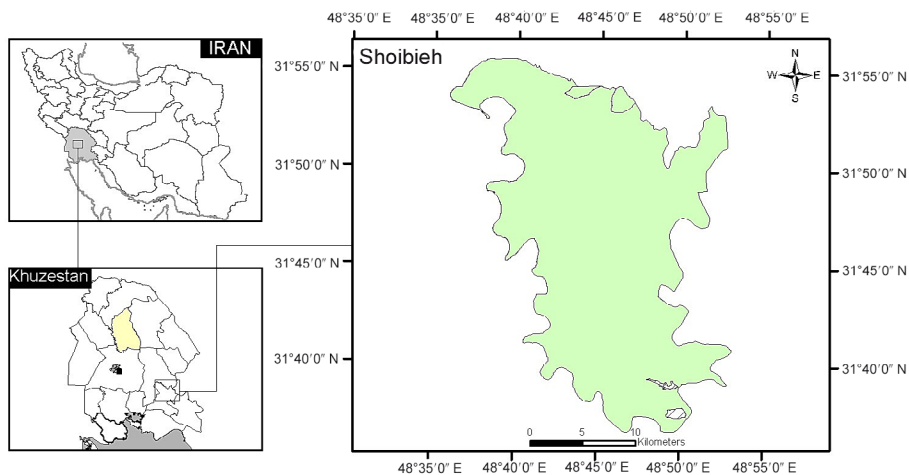


Fig. 1. Local map of the study area

of Ahwaz, 31° 36' to 31° 54' N and 48° 37' to 48° 53' E. The average annual temperature and precipitation for the period of 1959–2009 were 25.30 C° and 335.70 mm, respectively. Also, the annual Potential Evapotranspiration (PET) of the area is 1755.82 mm (Table 1) (TORFI 2010). But, due to the irrigation water quality parameters (such as  $EC < 1 \text{ dS m}^{-1}$  and etc) are in good condition, the problem of high PET not influences high salinity of water, and limits the use of pressurized irrigation systems.

Table 1  
Mean air temperature, relative humidity and total monthly rainfall and evaporation (1959–2009) at Shushtar

Parameter	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Average
Temperature [°C]	12.80	14.50	18.50	24.20	30.20	34.50	37.20	37.00	33.90	27.90	20.80	15.00	25.30
Relative humidity [%]	72.00	66.20	53.40	42.10	28.30	22.20	23.30	25.10	25.90	34.90	52.70	68.70	42.90
Total Rainfall [mm]	69.30	44.00	61.50	28.30	8.70	0	0	0	0	6.50	27.10	90.30	335.70
Potential ET [mm]	28.20	33.80	62.83	110.56	208.73	272.08	272.52	267.81	223.03	143.9	86.42	45.94	1755.82

### Irrigation Schemes

The Karun River supplies the bulk of the water demands of the region. The application of irrigated agriculture has been common in the study area. Over much of the Shoibieh Plain, the use of surface irrigation systems has been applied for crops. The major irrigated crops grown in this area are wheat, barley, alfalfa and maize. There are very few instances of sprinkler and drip irrigation on large area farms in the Shoibieh Plain. Currently, the irrigation systems used by farmlands in the region are furrow irrigation, basin irrigation and border irrigation schemes.

### Soil Sampling and Analyses

The area is composed of three distinct physiographic features i.e. River Alluvial Plains and Plateaux and Terraces, of which the River Alluvial Plains physiographic unit is the dominating feature. Also, twelve different soil series were found in the area (Table 2). The semi-detailed soil survey report of the Shoibieh Plain (ALBAJI 2008) was used in order to determine the soil characteristics. Table 3 has shown some of physico-chemical characteristics for reference profiles of different soil series in the plain. The land evaluation was determined

based upon topography and soil characteristics of the region (ALBAJI et al. 2009b). The topographic characteristics included slope and soil properties such as soil texture, depth, salinity, drainage and calcium carbonate content were taken into account (BEHZAD et al. 2009). Soil properties such as cation exchange capacity (CEC), percentage of basic saturation (PBC), organic matter (OM) and pH were considered in terms of soil fertility (SYS et al. 1991). SYS et al. (1991) suggested that soil characteristics such as OM and PBS do not require any evaluation in arid regions whereas clay CEC rate usually exceeds the plant requirement without further limitation, thus, fertility properties can be excluded from land evaluation if it is done for the purpose of irrigation. According to the particular semi-detailed studies of the region, samples were taken from each soil series profiles and laboratory analysis were carried out based upon the conventional methods of the Iranian Soil and Water Research

Table 2

Soil series of the study area

Series No.	Characteristics description
1	soil texture „medium: L”, slight salinity and alkalinity limitation, depth 130 cm, gently sloping: 2 to 5% well drained
2	soil texture „medium: SL”, without salinity and alkalinity limitation, depth 150 cm, level to very gently sloping: 0 to 2%, well drained
3	soil texture „medium: SL”, without salinity and alkalinity limitation, depth 60 cm, gently sloping: 2 to 5%, well drained
4	soil texture „medium: SL”, without salinity and alkalinity limitation, depth 160 cm, gently sloping: 2 to 5%, well drained
5	soil texture „medium: SIL”, slight salinity and alkalinity limitation, depth 135 cm, level to very gently sloping: 0 to 2%, well drained
6	soil texture „heavy: SICL”, without salinity and alkalinity limitation, depth 140 cm, level to very gently sloping: 0 to 2%, well drained
7	soil texture „heavy: SICL”, very without salinity and alkalinity limitation, depth 150 cm, level to very gently sloping: 0 to 2% well drained
8	soil texture „heavy: SCL”, without salinity and alkalinity limitation, depth 150 cm, level to very gently sloping: 0 to 2% well drained
9	soil texture „heavy: SICL”, slight salinity and alkalinity limitation, depth 130 cm, level to very gently sloping: 0 to 2% well drained
10	soil texture „heavy: SICL”, severe salinity and alkalinity limitation, depth 140 cm, level to very gently sloping: 0 to 2% moderately drained
11	soil texture „medium: SIL”, without salinity and alkalinity limitation, depth 150 cm, level very gently sloping: 0 to 2% moderately drained
12	soil texture „very heavy: SIC”, without salinity and alkalinity limitation, depth 140 cm, level to very gently sloping: 0 to 2%, imperfectly drained

Texture symbols: SL – sandy loam; L – loam; SCL – sandy clay loam; SICL – silty clay loam; SIL – silty loam; SIC – silty clay.

Table 3

Some of physico-chemical characteristics for reference profiles of different soil series

Soil series No.	Soil series name	Depth [cm]	Soil texture	ECe [ds m <sup>-1</sup> ]	pH	OM [%]	CEC [meq/100 g]	CaCO <sub>3</sub> [%]
1	Qhaleh Khan	130	L	4.10	7.60	0.62	12.36	43.00
2	Sheykh Hossein	150	SL	1.50	7.50	0.14	11.92	42.00
3	Bala	60	SL	3.20	7.90	0.24	10.54	44.00
4	Kakoli	160	SL	0.50	8.20	0.08	11.47	51.00
5	Abbasieh	135	SIL	5.90	7.60	0.39	12.73	44.00
6	Karun	140	SICL	1.00	7.60	0.41	10.22	51.00
7	Deylam	150	SICL	2.90	7.50	0.32	10.81	49.00
8	Qalimeh	150	SCL	1.50	7.90	0.26	12.05	49.00
9	Abdul Amir	130	SICL	7.50	7.80	0.57	10.38	46.00
10	Khoshmakan	140	SICL	31.00	7.60	0.20	10.87	44.00
11	Karkheh	150	SIL	1.90	7.70	0.37	9.26	50.00
12	Dez	140	SIC	3.50	7.60	0.39	10.64	40.00

Institute (JALALI 1997, MAHJOobi et al. 2010), and the following properties were measured by due methods: electrical conductivity by conductivity meter (inoLab, Con Level 1, WTW), soil texture by agitator and hydrometer and lime settlement rate by titration method (PAGE et al. 1992).

The groups of soils that had similar properties and were located in a same physiographic unit were categorized as soil series and were classified to form a soil family as per the *Soil Survey Staff* (2008). Ultimately, twelve soil series were selected for the surface, sprinkler and drip irrigation land suitability. Twelve soil series and forty nine series phases were derived from the semi-detailed soil study of the area. The soil series are shown in Figure 2 as the basis for further land evaluation practice. The soils of the area are of Aridisols and Entisols orders (*Soil Survey Staff*. 2008). Also, the soil moisture regime is Aridic while the soil temperature regime is Hyperthermic (ALBAJI 2008).

In order to obtain the average soil texture, salinity and CaCO<sub>3</sub> for the upper 150 cm of soil profile, the profile was subdivided into 6 equal sections and weighting factors of 2, 1.5, 1, 0.75, 0.50 and 0.25 were used for each section, respectively (Sys et al., 1991). Due to topsoil (0–75 cm) is important than subsoil (75–150 cm) for plant growth (plant nutrition, water absorption and etc), thus the weighting factors of topsoil (2, 1.5, 1) are bigger than subsoil (0.75, 0.50, 0.25).



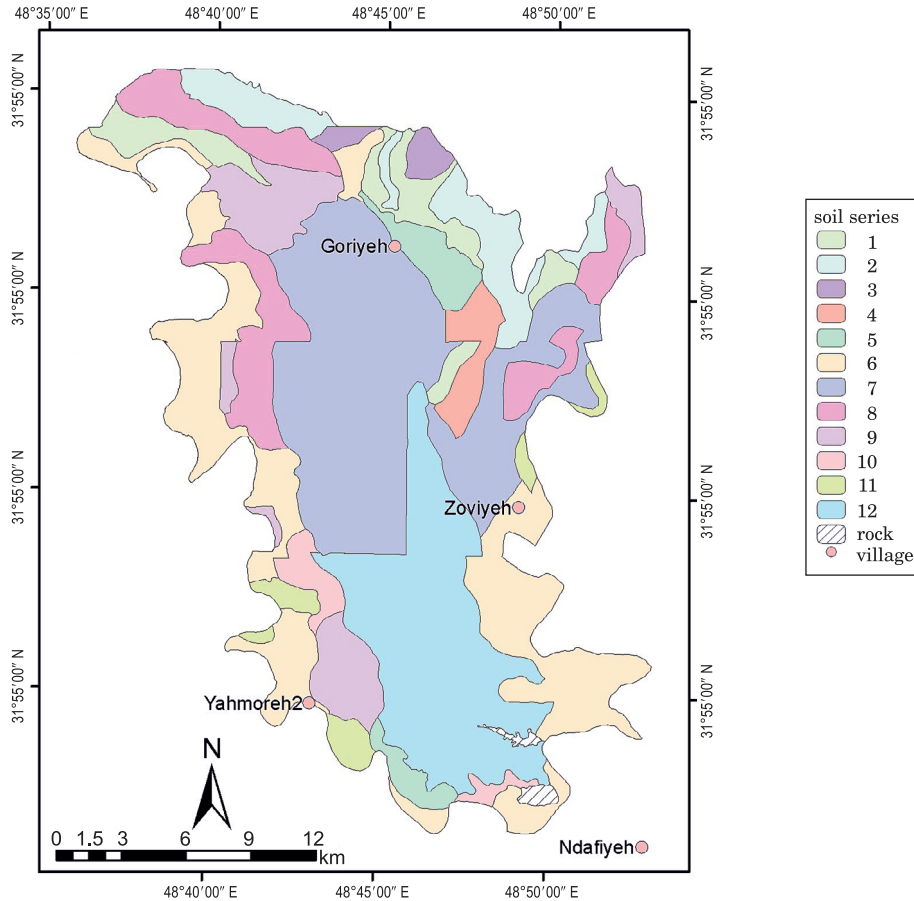


Fig. 2. Soil map of the study area

For the evaluation of land suitability for surface, sprinkler and drip irrigation, the parametric evaluation system was used (SYS et al. 1991). This method is based on morphology, physical and chemical properties of soil. The method, the land is evaluated according to numerical indexes. In this classification system, firstly a degree, whose rate is from 0 to 100, is given to any land characteristic through comparing them with the tables of soil requirements. The specified degrees are used in order to measure the land index that is a multiplicative index that combines ratings assigned to soil map units and other physical conditions that affect the land use (OLSEN 1981).

The chemical and physical soil proprieties are determined in the soil laboratory of Khuzestan Water and Power Authority using different kinds of analyses processing (ALBAJI 2008). This approach allows a calculation

Table 4  
Rating of textural classes for irrigation

Tex	Rating for surface irrigation				Rating for sprinkler irrigation				Rating for drip irrigation			
	fine gravel [%]		coarse gravel [%]		fine gravel [%]		coarse gravel [%]		fine gravel [%]		coarse gravel [%]	
	< 15	15-40	40-75	15-40	40-75	15-40	40-75	15-40	40-75	15-40	40-75	15-40
CL	100	90	80	80	50	50	50	100	100	90	80	80
SiL	100	90	80	80	50	50	50	100	100	90	80	80
SCL	95	85	75	75	45	45	45	95	95	85	75	75
L	90	80	70	70	45	45	45	90	90	80	70	70
SiL	90	80	70	70	45	45	45	90	90	80	70	70
Si	90	80	70	70	45	45	45	90	90	80	70	70
SiC	85	95	80	80	40	40	40	85	85	95	80	80
C	85	95	80	80	40	40	40	85	85	95	80	80
SC	80	90	75	75	35	35	35	95	95	90	85	80
SL	75	65	60	60	35	35	35	90	95	85	80	75
LS	55	50	45	45	25	25	25	70	65	85	75	60
S	30	25	25	25	25	25	25	50	45	70	65	35

Tex – textural classes: CL – clay loam; SiL – silty loam; SCL – sandy clay loam; L – loam; SiL – silty loam; SiC – silty clay; C – clay;  
 SC – sandy clay; SL – sandy loam; LS – loamy sand; S – sandy

of a suitability index for irrigation considering some factors influencing the soil suitability. These factors are (SYS et al. 1991):

- soil texture: rated taking in account the permeability and available water content, and calculated, as weighted average, for the upper 100 cm;
- soil depth: rated with regard to the thickness and the characteristic of the soil layers (horizons);
- calcium carbonate content: influencing the relationship between soil and water, and the availability of nutrient supply for plant (150 cm of soil profile). It is rated with regard to the  $\text{CaCO}_3$  content effect on soil profile;
- salinity: rated on the base of the electrical conductivity of soil solution;
- drainage: a limiting factor when it is imperfect or weak. The rating for drainage is related to texture;
- slope: estimated considering the difference between terraced and non-terraced slopes.

These factors (including soil texture, soil depth, calcium carbonates status, electrical conductivity of soil solution, drainage properties and slope) were also considered and values were assigned to each factors as per the related tables (Tables 4–9) [SYS et al. (1991) for surface and drip irrigation; ALBAJI (2010a) for sprinkler irrigation].

Thus, the capability index for irrigation ( $C_i$ ) was developed as shown in the equation below:

$$C_i = A \cdot \frac{B}{100} \cdot \frac{C}{100} \cdot \frac{D}{100} \cdot \frac{E}{100} \cdot \frac{F}{100}$$

where:

- $A$  – rating of soil texture;
- $B$  – rating of soil depth;
- $C$  – rating of calcium carbonate content;
- $D$  – rating of electrical conductivity;
- $E$  – rating of soil drainage;
- $F$  – rating of soil slope.

Table 5

Rating of soil depth for irrigation

Soil depth [cm]	Rating for surface irrigation	Rating for sprinkler irrigation	Rating for drip irrigation
< 20	25	30	35
20–50	60	65	70
50–80	80	85	90
80–100	90	95	100
> 100	100	100	100

Table 6

Rating of  $\text{CaCO}_3$  for irrigation

$\text{CaCO}_3$ [%]	Rating for surface irrigation	Rating for sprinkler irrigation	Rating for drip irrigation
<0.3	90	90	90
0.3–10	95	95	95
10–25	100	100	95
25–50	90	90	80
>50	80	80	70

Table 7

Rating of salinity for irrigation

EC [ds $\text{m}^{-1}$ ]	Rating for surface irrigation		Rating for sprinkler irrigation		Rating for drip irrigation	
	C, SiC, SiCL, S, SC textures	other textures	C, SiC, SiCL, S, SC textures	other textures	C, SiC, SiCL, S, SC textures	other textures
< 4	100	100	100	100	100	100
4–8	90	95	95	95	95	95
8–16	80	50	85	50	85	50
16–30	70	30	75	35	75	35
> 30	60	20	65	20	65	25

C – clay; SiC – silty clay; SiCL – silty clay loam; S – sand; SC – sandy clay

Table 8

Rating of drainage classes for irrigation

Drainage classes	Rating for surface irrigation		Rating for sprinkler irrigation		Rating for drip irrigation	
	C, SiC, SiCL, S, SC textures	other textures	C, SiC, SiCL, S, SC textures	other textures	C, SiC, SiCL, S, SC textures	other textures
Well drained	100	100	100	100	100	100
Moderately drained	80	90	90	95	100	100
Imperfectly drained	70	80	75	85	80	90
Poorly drained	60	65	65	70	70	80
Very poorly drained	40	65	45	65	50	65
Drainage status not known	70	80	70	80	70	80

C – clay; SiC – silty clay; SiCL – silty clay loam; S – sand; SC – sandy clay

Table 9

## Rating of slope for irrigation

Slope classes [%]	Rating for surface irrigation		Rating for sprinkler irrigation		Rating for drip irrigation	
	non-terraced	terraced	non-terraced	terraced	non-terraced	terraced
0–1	100	100	100	100	100	100
1–3	95	95	100	100	100	100
3–5	90	95	95	100	100	100
5–8	80	90	85	95	90	100
8–16	70	80	75	85	80	90
16–30	50	65	55	70	60	75
> 30	30	45	35	50	40	55

In Table 10 the ranges of capability index and the corresponding suitability classes are shown.

Table 10

Suitability classes for the irrigation capability indices ( $C_i$ ) classes

Capability index	Definition	Symbol
> 80	highly suitable	$S_1$
60–80	moderately suitable	$S_2$
45–59	marginally suitable	$S_3$
30–44	currently not suitable	$N_1$
< 29	permanently not suitable	$N_2$

## Land Suitability Maps

In order to develop land suitability maps for different irrigation methods, a semi-detailed soil map (Figure 2) prepared by Albaji was used, and all the data for soil characteristics were analyzed and incorporated in the map using ArcGIS 9.2 software.

The digital soil map base preparation was the first step towards the presentation of a GIS module for land suitability maps for different irrigation systems. The soil map was then digitized and a database prepared. A total of twelve different polygons or soil series were determined in the base map. Soil characteristics were also given for each soil series. These values were used to generate the land suitability maps for surface, sprinkler and drip irrigation

systems using Geographic Information Systems. In Figure 3 schematic chart of GIS application for land suitability map for different Irrigation methods is shown.

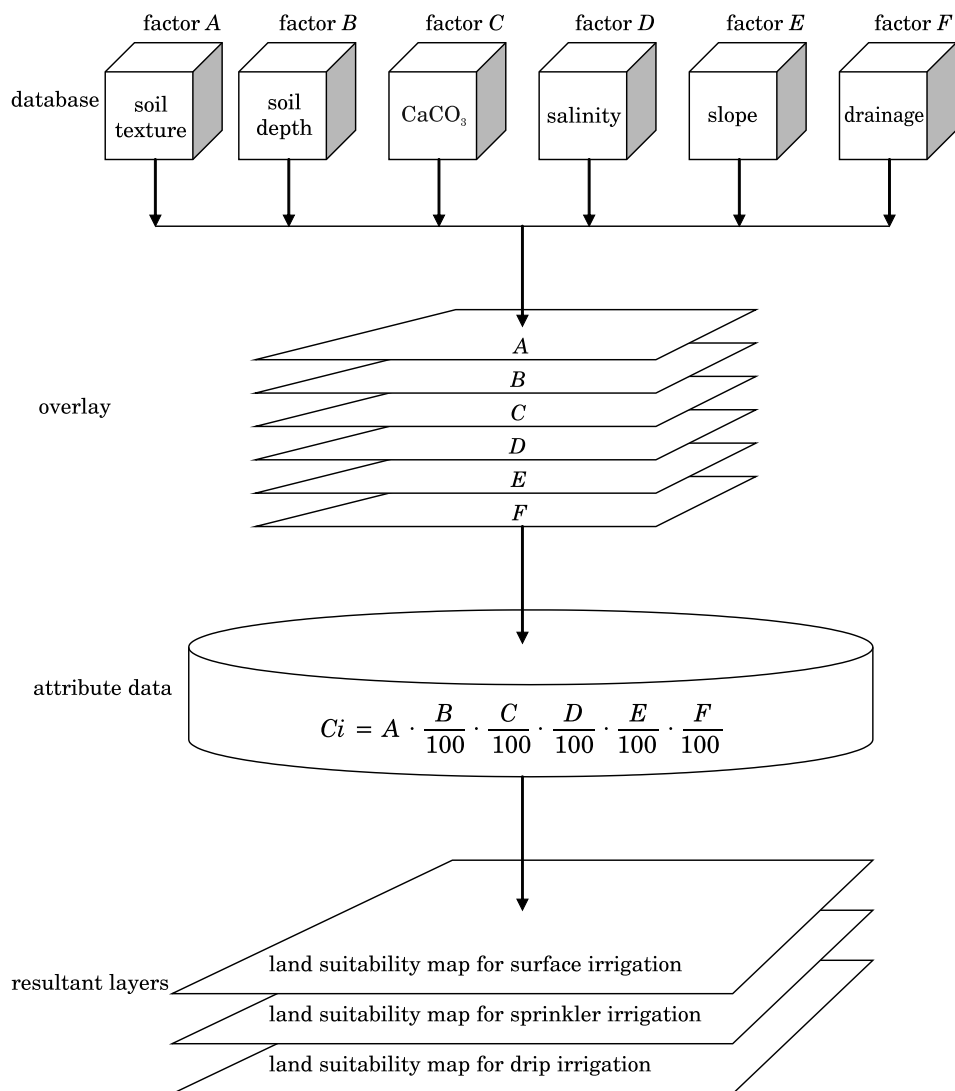


Fig. 3. Schematic chart of GIS application for land suitability map for different irrigation methods

## Results and Discussion

As shown in Table 11 and Table 12 for surface irrigation, the soil series coded 5, 7 and 8 (18 804.64 ha – 37.9%) were highly suitable ( $S_1$ ; Land having no, or insignificant limitations for irrigation); soil series coded 1, 2, 6, 9 and 11 (19 773.54 ha – 39.8%) were classified as moderately suitable ( $S_2$ ; Land having minor limitations for irrigation), soil series coded 3, 4 and 12 (9925.16 ha – 20%) were found to be marginally suitable ( $S_3$ ; land having moderate limitations for irrigation) and only soil series coded 10 (884.62 ha – 1.8%) were classified as currently not-suitable ( $N_1$ ; land having severe limitations for irrigation) for any surface irrigation practices. There was no permanently not suitable land ( $N_2$ ; land that have so severe limitations for irrigation) in this plain.

The analysis of the suitability irrigation maps for surface irrigation (Figure 4), indicate that the large parts of the cultivated area in this plain (located in the center and the north) is deemed as being highly suitable land due to deep soil, good drainage, texture, salinity and proper slope of the area. The moderately suitable lands could be observed over the largest portion of the plain (west, east, center and south parts) due to the medium calcium carbonate content limitations (It is limitations due to amount of  $CaCO_3$  of soil. If the soil's

Table 11  
 $C_i$  values and suitability classes of surface, sprinkler and drip irrigation for each soil series

Codes of soil series	Surface irrigation		Sprinkler irrigation		Drip irrigation	
	$C_i$	suitability classes	$C_i$	suitability classes	$C_i$	suitability classes
1	71.17	$S_{2s}$	75.02	$S_{2s}$	68.4	$S_{2s}$
2	65.81	$S_{2s}$	81	$S_1$	76	$S_{2s}$
3	49.95	$S_{3s}$	67.12	$S_{2s}$	68.4	$S_{2s}$
4	55.5	$S_{3s}$	70.2	$S_{2s}$	66.5	$S_{2s}$
5	83.36	$S_1$	85.5	$S_1$	76	$S_{2s}$
6	78	$S_{2s}$	80	$S_1$	70	$S_{2s}$
7	87.75	$S_1$	90	$S_1$	80	$S_1$
8	83.36	$S_1$	85.5	$S_1$	76	$S_{2s}$
9	78.97	$S_{2sn}$	85.5	$S_1$	76	$S_{2s}$
10	42.12	$N_{1snw}$	52.65	$S_{3snw}$	52	$S_{3sn}$
11	78.97	$S_{2sw}$	85.5	$S_1$	80	$S_1$
12	52.21	$S_{3sw}$	57.37	$S_{3sw}$	54.4	$S_{3sw}$

Limiting factors for surface irrigation: s – calcium carbonate; w – drainage.

Limiting factors for sprinkler and drip irrigations: s – calcium carbonate.

Table 12

Distribution of surface, sprinkler and drip irrigation suitability

Suitability	Surface irrigation			Sprinkler irrigation			Drip irrigation		
	soil series	area [ha]	ratio [%]	soil series	area [ha]	ratio [%]	soil series	area [ha]	ratio [%]
S <sub>1</sub>	5, 7, 8	18804.64	37.9	2, 5, 6, 7, 8, 9, 11	36046.49	72.6	7, 11	13584.85	27.3
S <sub>2</sub>	1, 2, 6, 9, 11	19773.54	39.8	1, 3, 4	4151.37	8.4	1, 2, 3, 4, 5, 6, 8, 9	26613.01	53.7
S <sub>3</sub>	3, 4, 12	9925.16	20	10, 12	9190.1	18.5	10, 12	9190.1	18.5
N <sub>1</sub>	10	884.62	1.8	–	–	–	–	–	–
N <sub>2</sub>	–	–	–	–	–	–	–	–	–
Mis land	–	234.09	0.5	–	234.09	0.5	–	234.09	0.5
Total	–	49 622.04	100	–	49 622.04	100	–	49 622.04	100

Miscellaneous land – hill, sand dune and river bed

CaCO<sub>3</sub> was between 25–50%, the limitation of calcium carbonate is medium for surface irrigation) and medium drainage limitations (It is limitations due to a ground water table. if ground water table located between 2 and 3 m, the limitation of drainage is medium). Other factors such as slope, depth, salinity and alkalinity have no influence on the suitability of the area whatsoever. The map also indicates that only some part of the cultivated area in this plain was evaluated as marginally suitable because of the high calcium carbonate content. The current non-suitable lands covered the smallest part of the plain. Because of high content of calcium carbonate and very sever drainage, salinity and alkalinity limitations. There was no permanently non-suitable land in this plain. For almost the total study area elements such as soil depth, soil texture and slope were not considered as limiting factors.

In order to verify the possible effects of different management practices, the land suitability for sprinkler and drip irrigation was evaluated (Table 11 and Table 12).

For sprinkler irrigation, soil series coded 2, 5–9 and 11 (36 046.49 ha – 72.6%) were highly suitable (S<sub>1</sub>) while soil series coded 1, 3 and 4 (41 51.37 ha- 8.4%) were classified as moderately suitable (S<sub>2</sub>). Further, soil series coded 10 and 12 (9190.1 ha – 18.5%) were found to be marginally suitable (S<sub>3</sub>) for sprinkler irrigation.

Regarding sprinkler irrigation (Figure 5), the highly suitable area can be observed in the largest part of the cultivated zone in this plain (located in the north, center, west and the east) due to deep soil, good drainage, texture,



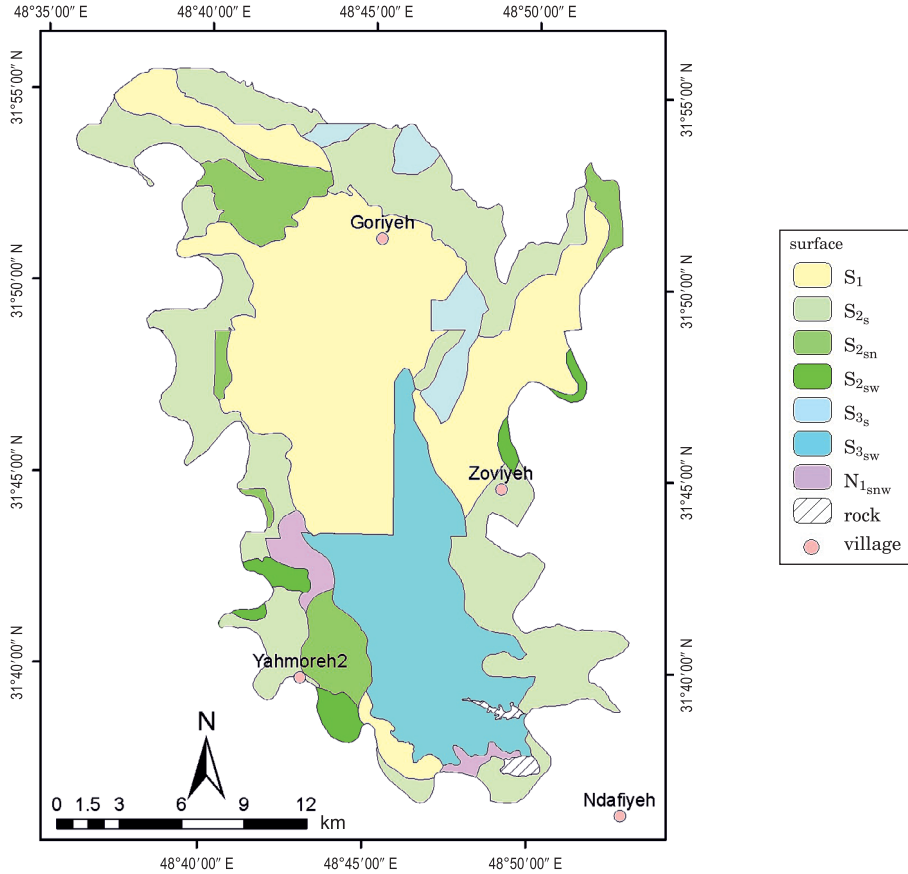


Fig. 4. Land suitability map for surface irrigation:  $S_1$ ,  $S_{2s}$ ,  $S_{2sn}$ ,  $S_{2sw}$  – highly suitable;  $S_{3s}$ ,  $S_{3sw}$  – marginally suitable;  $N_{1snw}$  – currently not suitable

salinity and proper slope of the area. As seen from the map, the smallest part of the cultivated area in this plain was evaluated as moderately suitable for sprinkler irrigation because of the medium calcium carbonate content. Other factors such as drainage, depth, salinity and slope never influence the suitability of the area. The marginally suitable lands were found only in the center and south of the area studied. The limiting factors for these soil series were high content of calcium carbonate and severe limitations of drainage, salinity and alkalinity. The current non-suitable lands and permanently not-suitable lands did not exist in this plain. For almost the entire study area slope, soil depth and soil texture were not as limiting factors.

For drip irrigation, soil series coded 7 and 11 (13 584.85 ha – 27.3%) were highly suitable ( $S_1$ ) while soil series coded 1–9 (26 613.01 ha – 53.7%) were

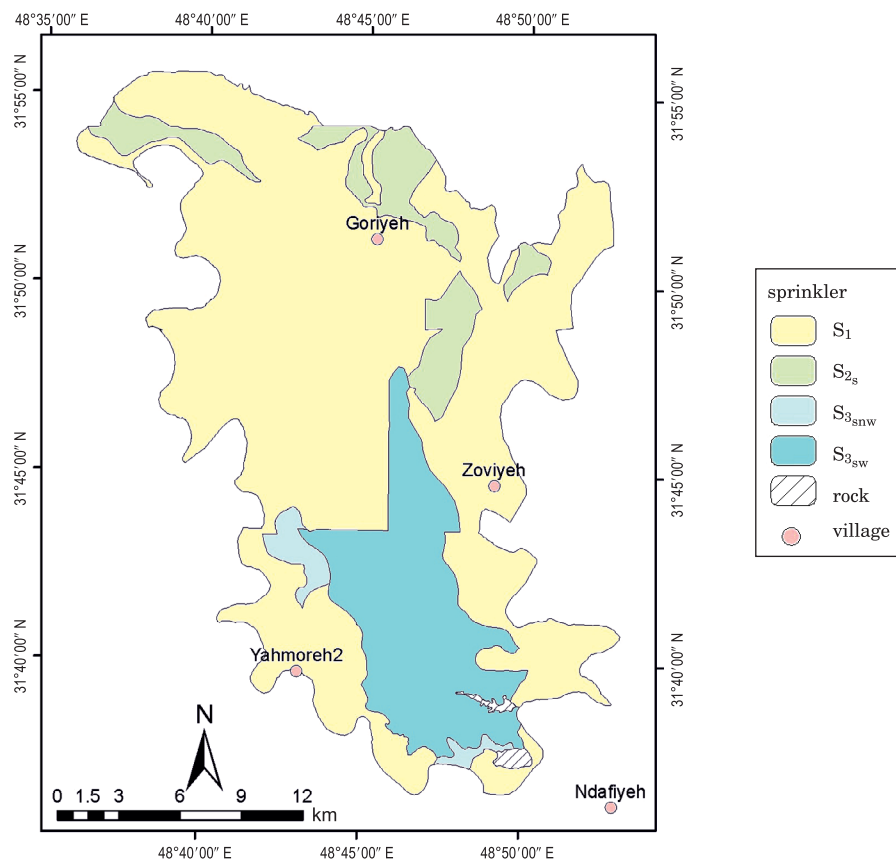


Fig. 5. Land suitability map for sprinkler irrigation: S<sub>1</sub> – highly suitable; S<sub>2s</sub> – moderately suitable; S<sub>3snw</sub>, S<sub>3sw</sub> – marginally suitable

classified as moderately suitable (S<sub>2</sub>) and further, soil series coded 10 and 12 (9190.1 ha, 18.5%) were found to be slightly suitable (S<sub>3</sub>) for drip irrigation. In this case (Figure 6), the highly suitable area can be observed in the some part of the cultivated zone located in the center and east area. They were due to deep soil, good drainage, suitable texture, salinity and proper slope of the area. The largest portion of the cultivated area in the plain was evaluated as moderately suitable for drip irrigation; because of the medium calcium carbonate content. The map also indicated that the smallest portion of the cultivated area in this plain which is located in the south and center of the zone was evaluated as marginally suitable; due to the high calcium carbonate content and severe drainage, salinity and alkalinity limitations. The current non-suitable lands and permanently not-suitable lands do not exist in this plain. For almost the entire study area slope, soil depth and soil texture were not as limiting factors.

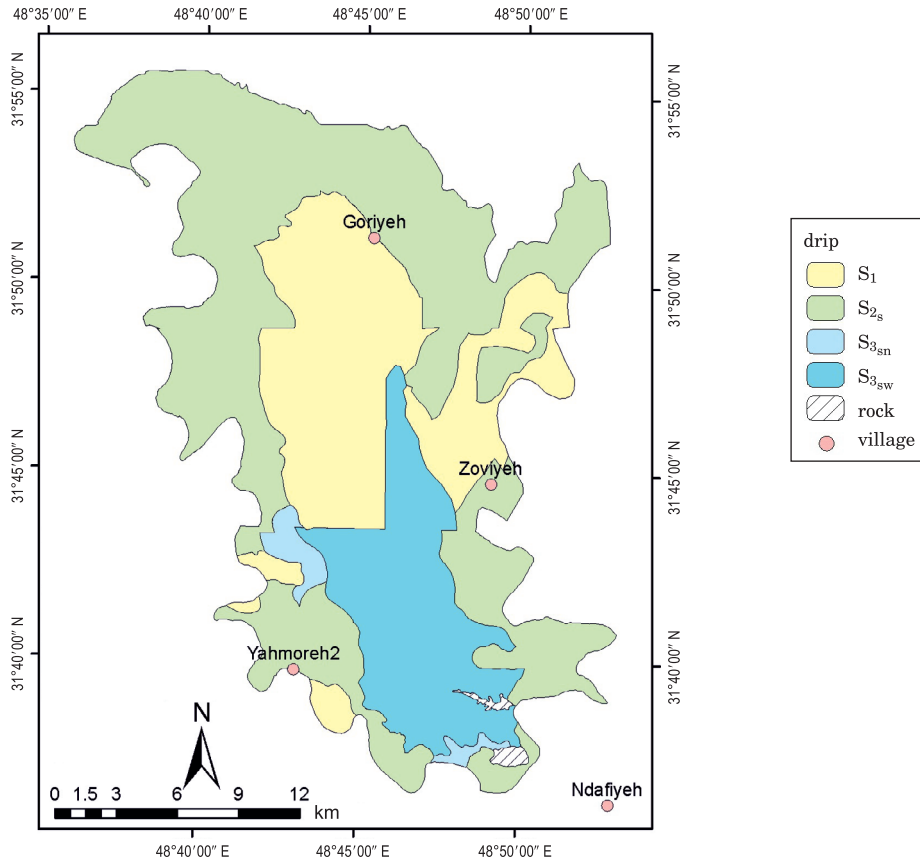


Fig. 6. Land Suitability Map for Drip Irrigation :  $S_1$  – highly suitable;  $S_{2s}$  – moderately suitable;  $S_{3sn}$ ,  $S_{3sw}$  – marginally suitable

The comparison of the capability indexes for surface, sprinkler and drip irrigation (Table 11 and Table 13) indicated that in soil series coded 3 applying drip irrigation systems was the most suitable option as compared to surface and sprinkler irrigation systems. In soil series coded 1–12 applying sprinkler irrigation systems was more suitable than surface and drip irrigation systems. Figure 7 shows the most suitable map for surface, sprinkler and drip irrigation systems in the Shoibieh Plain as per the capability index ( $C_i$ ) for different irrigation systems. As seen from this map, the largest part of this plain was suitable for sprinkler irrigation systems and very small parts of this area was suitable for drip irrigation systems.

Table 13

The most suitable soil series for surface, sprinkler and drip irrigation systems by notation to capability index ( $C_i$ ) for different irrigation systems

Codes of soil series	The maximum capability index for irrigation ( $C_i$ )	Suitability classes	The most suitable irrigation systems	Limiting factors
1	75.02	$S_{2s}$	sprinkler	S
2	81	$S_1$	sprinkler	no exist
3	68.4	$S_{2s}$	drip	S
4	70.2	$S_{2s}$	sprinkler	S
5	85.5	$S_1$	sprinkler	no exist
6	80	$S_1$	sprinkler	no exist
7	90	$S_1$	sprinkler	no exist
8	85.5	$S_1$	sprinkler	no exist
9	85.5	$S_1$	sprinkler	no exist
10	52.65	$S_{3snw}$	sprinkler	SNW
11	85.5	$S_1$	sprinkler	no exist
12	57.37	$S_{3sw}$	sprinkler	SN

Limiting factors for sprinkler irrigations: S – calcium carbonate; N – salinity & alkalinity; W – drainage.

Limiting factors for drip irrigation: S – calcium carbonate

The comparison between different irrigation systems (surface and pressurized systems) shows a big difference in the suitability of the different irrigation methods. Pressurized irrigation systems (sprinkler and drip irrigation systems) can be a good irrigation method, if properly managed (good planning, use of filters, etc) (BAVI et al. 2009, NASERI et al. 2009, ALBAJI et al. 2010b, ALBAJI et al. 2010c, DIOUF-SARR 2011, ALBAJI-HEMADI 2011, JOVZI et al. 2012, ALBAJI et al. 2013).

The results of Table 11 and Table 13 indicated that by applying sprinkler irrigation instead of surface and drip irrigation methods, the land suitability of 48 805, 43 ha (98.3%) of the Shoibieh Plain's land could be improved substantially. However by applying drip irrigation instead of surface and sprinkler irrigation methods, the suitability of 582, 53 ha (1.2%) of this Plain's land could be improved. The comparison of the different types of irrigation revealed that sprinkler irrigation was more effective and efficient than the drip and surface irrigation methods and improved land suitability for irrigation purposes. Moreover, the main limiting factors in using surface irrigation methods in this area were calcium carbonate content and drainage and the main limiting factor in using sprinkler and drip irrigation methods in this area was calcium carbonate content.

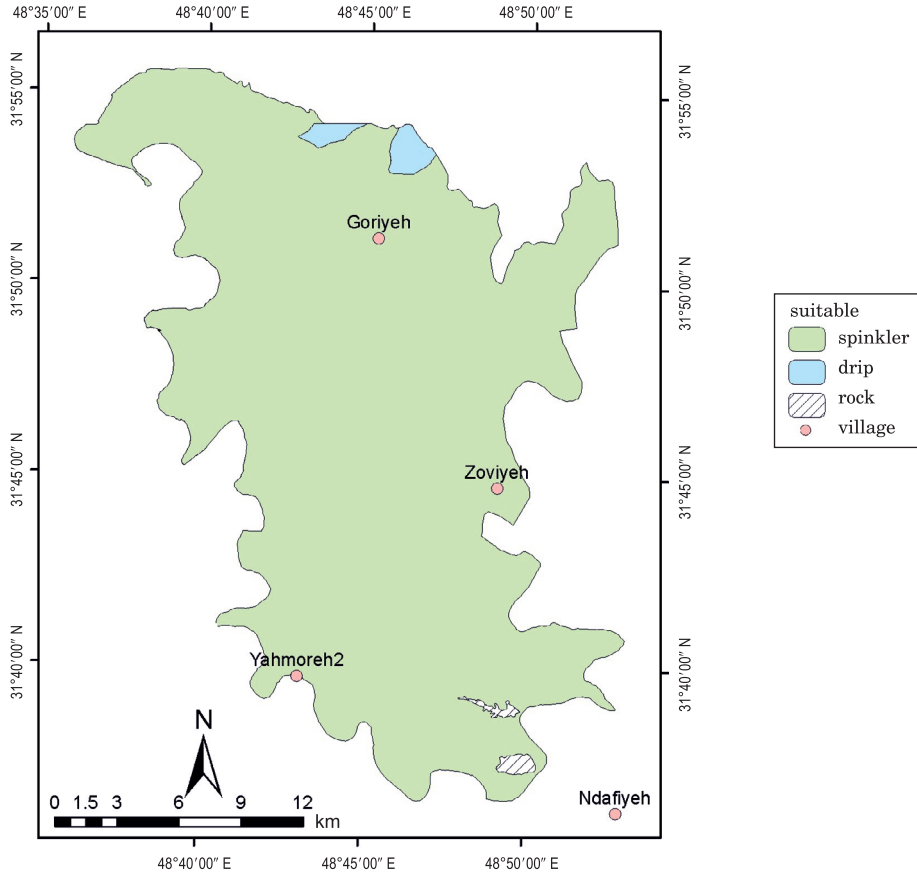


Fig. 7. The most suitable map for different irrigation systems

## Conclusions

Several parameters were used for the analysis of the field data in order to compare the suitability of different irrigation systems. The analyzed parameters included soil and land characteristics. The results obtained showed that sprinkler irrigation systems are more suitable than drip and surface irrigation methods for most of the study area. The major limiting factor for both sprinkler and drip irrigation methods was soil calcium carbonate content. However for surface irrigation method, soil calcium carbonate content and drainage were restricting factors. The results of the comparison between the maps indicated that the introduction of a different irrigation management policy would provide an optimal solution in as such that the application of

sprinkler irrigation technique could provide beneficial and advantageous. This is the current strategy adopted by large companies cultivating in the area and it will provide to be economically viable for Farmers in the long run.

Such a change in irrigation management practices would imply the availability of larger initial capitals to farmers (different credit conditions, for example) as well as a different storage and market organization.

In this study, an attempt has been made to analyze and compare three irrigation systems by taking into account various soil and land characteristics. The results obtained showed that sprinkler irrigation methods are more suitable than drip and surface irrigation methods for most of the soils tested. Moreover, because of the insufficiency of surface and ground water resources, and the aridity and semi-aridity of the climate in this area, sprinkler irrigation methods are highly recommended for a sustainable use of this natural resource; hence, the changing of current irrigation methods from gravity (surface) to pressurized (sprinkler) in the study area are proposed.

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## MULTISENSORY VALORIZATION OF A LANDSCAPE AS A METHOD FOR IDENTIFYING AREAS REQUIRING VALUE. A CASE STUDY

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**Key words:** bonitation, landscape, center west Poland, sensorial stimuli, vicinity of lake.

### Abstract

Multisensory landscape valorization as used in this study was intended to identify areas that required value. Our unique approach to the methodology allowed a comprehensive analysis of the research area, i.e. Lake Rusałka in Poznań, a major city in central western Poland. Analysis was based on recording multisensory experiences and assigning bonitation points to stimuli, enabling a comprehensive assessment of the analyzed area. The thusly designated zones indicated sites which required technical improvements and adequate planning in order to improve their perception.

### MULTISENSORYCZNA WALORYZACJA KRAJOBRAZU JAKO METODA WYODRĘBNIANIA STREF WYMAGAJĄCYCH DOWARTOŚCIOWANIA. STUDIUM PRZYPADKU

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**Słowa kluczowe:** bonitacja, krajobraz, śródkowozachodnia Polska, bodźce zmysłowe, otoczenie jeziora.

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### Abstrakt

Multisensoryczna waloryzacja krajobrazu ma na celu wyodrębnienie stref wymagających dowartościowania. Autorska modyfikacja podejść metodycznych pozwoliła na kompleksową analizę terenu badawczego. Stanowiło go jezioro Rusalka w Poznaniu w środkowozachodniej Polsce. Waloryzację oparto na odbiorze wrażeń wszystkimi zmysłami. Przeprowadzenie procesu w sposób multisensoryczny wraz z bonitacją punktową przypisaną odpowiednim bodźcom umożliwiło całościową i kompleksową ocenę analizowanego terenu. Wyznaczone w ten sposób strefy wskazały miejsca, które potrzebują zabiegów technicznych i planistycznych do poprawienia ich odbioru.

## Introduction

Ambiguity is a most adequate term for describing a landscape. It is the common denominator of all definitions that have been constructed over the centuries (ZUBE et al. 1982, *Europejska...* 2000, MYGA-PIĄTEK 2001, *Ocena i wycena...* 2007, JANZ 2010, MAZURSKI 2012). But nowadays we may observe that the aesthetics of a landscape is becoming increasingly important in modern society (PORTEOUS 2004) and accordingly we have observed a growing number of projects aimed at revitalizing various areas through technical improvements and planning (ANIČIĆ et al. 2007, HUZUI et al. 2011, JASZCZAK and DREKSLER 2011, KAČIČ and LIDÉN 2011).

It is worth emphasizing that a perception of a landscape is multisensory (PORTEOUS 1985, PORTEOUS and MASTIN 1985, CARLES et al. 1999, *Landscapes...* 2003, PIETRZAK 2008). According to KOWALCZYK (1992), this concept means that the perception of surroundings involves vision, hearing, smell and feeling. Landscapes influence us in many aspects, creating positive or negative feelings which affect the individual assessment (valorization) of the landscape (WOJCIECHOWSKI 1986, 1994).

Valorization is a subjective term which can, similar to the notion of landscape itself, also be considered quite ambiguous (DAKIN 2003, KISTOWSKI 2006, SUROVA and PINTO-CORREIA 2008, STEPHENSON 2010, BATISTA et al. 2012). First of all it consists in defining problems and opportunities in the analyzed landscape. A thorough and careful approach makes it possible to eliminate the existing threats and to develop opportunities for the area that would ensure sustainable development and spatial order that is so much in demand in modern times (RASZEJA 2005, VIZZARI 2011, MIRSANJARI and MIRSANJARI 2012, HELDAK and RASZKA 2013).

In this study we perform valorization of the most neglected areas in the vicinity of Lake Rusalka in Poznań, which should enable the recovery of the area.

## Materials and Methods

The research involved the immediate surroundings of Lake Rusałka, located in the center of Poznań, a major city (580,000 people) in the central western part of Poland (Figure 1). The lake is an artificial body of water, previously a clay excavation site used for the production of bricks. It was created by damming of the Bogdanka River in 1943 to an average depth of about 1.9 m (maximum depth of 9.0 m), covering an area of 36.7 hectares and 3330 m of shoreline.



Fig. 1. Location of the study area

Lake Rusałka is a popular recreational site, often frequented by city residents, especially in the summer. Its popularity has increased since 2011, when bathing opportunities were limited at another lake in Poznań, i.e. Lake Strzeszyńskie (JONIAK et al. 2013). On the northern shore of Lake Rusałka is a patrolled swimming area which can accommodate 10,000 people (according to data from the Poznań City Hall). Such a large concentration of people in one place reduces the comfort of visitors, which is additionally exacerbated by the excessive wear or even a lack of adequate infrastructure around the lake.

In order to determine sites that require value, we performed field tests involving an inventory of the current state of development, and the designation of areas with different aesthetic values based on our unique modification of multisensory landscape assessment. This modification was based on the combination of two methodologies, i.e. by SKARŻYŃSKI (1992) and KOWALCZYK (1992).

The first method (SKARŻYŃSKI 1992) was based on the assessment of landscape aesthetics perceived only visually, taking into account the basic conditions of the assessment. Its author distinguished five sets of criteria for landscapes, with a maximum of 5 points and a minimum of -2 points, which can be assigned to the criteria. In this paper, Skarżyński's method (1992) was modified by introducing a more varied scoring. It was assumed that the assessment of aesthetics and order is definitely most important and affects the sense of sight more strongly than other factors. The score ranges were increased, to (-10, +10) in group III for the assessment of natural sites and man-made objects, and to (-5, +10) in group IV for the sense of harmony. A slight modification was introduced in the number of landscape elements in the 2nd group of criteria.

The approach of KOWALCZYK (1992) focused on using a variety of methods to examine selected landscape elements, including the analysis of scientific literature to identify types of multisensory landscapes and surveys. One of the most important parts of this landscape evaluation was selecting the appropriate type of multisensory landscape. The next step was to determine the suitability of the landscape for recreation. KOWALCZYK (1992) conducted this assessment using a method proposed by *Ocena krajobrazu...* 1991, based on the assessment of 12 features of a landscape and assigning them scores ranging from -2 to +3 points. In this paper a modified WYRZYKOWSKI method was used to evaluate the suitability of Lake Rusalka for recreation.

This modification was based on a more accurate determination of the individual features of the landscape by assigning respective scores that corresponded to their intensity. In addition, some features covered by WYRZYKOWSKI (*Ocena krajobrazu...* 1991) were omitted as they overlapped criteria specified by SKARŻYŃSKI (1992). The first two properties, solar exposure and light, were combined into a joint category of sunlight with different degrees of intensity (Table 1). They were divided into two subgroups, i.e. open areas and closed areas. Another criterion was the number of perceived natural colors in the landscape. The next category were odors, perceived as positive or negative, with 3 degrees of intensity. Other categories included aeration, perceived humidity and noise.

Table 1 shows the separate categories and sub-categories, and scores from -10 to +10. The increased scale of points in the modification of the method by SKARŻYŃSKI (1992), involved categories that were assumed to have the greatest impact on the senses, i.e. smell and noise, the strongest factors of comfort for a visitor at a particular place. In order to obtain the most comprehensive assessment of the analyzed area which would combine the visual aspect with other senses, we combined both aforementioned methods. Assigning point scales to individual categories was used to reduce the subjectivity of the valorization, which was difficult to eliminate completely.

Table 1

Criteria of multisensory landscape characterization and point bonitation

Group	Criteria and their distribution	Point bonitation
1	2	3
I	Number of planes in the landscape: – three planes (and more) – two distinct planes with sporadic clearances of the third plane – two planes – one plane	5 4 3 1
II	Number of elements constituting the landscape and the possibility of their identification – varied landscape (> 5 elements) – moderately varied landscape (3-5 elements) – poor landscape (< 3 elements)	5 3 1
III	Variety of elements constituting a landscape and the possibility of their identification – water facilities dominant in the landscape noticeable (presence without domination) no water facilities – woody vegetation presence of dense woods and single trees or tree clusters presence of only a dense forest, individual trees or clusters shrubby vegetation no vegetation Individual anthropogenic objects or natural objects or their sets affecting the aesthetic value of the landscape – positive – neutral – negative – extremely negative	3 2 1 3 2 1 0 10 5 0 –5
IV	Co-occurrence of landscape elements (harmony) – harmonious landscape – landscape with partially disturbed harmony – landscape with strongly disturbed harmony – landscape with a completely disturbed harmony	10 5 –5 –10
V	The vertical structure of the landscape – well-developed – moderately developed – poorly developed	3 2 1
VI	Solar exposure of: – open areas high moderate low – areas enclosed by natural elements of the landscape high moderate low	5 3 1 1 3 5
	Dominant natural colors in the landscape > 2 natural colors	3

cont. Table 1

1	2	3
VII	2 natural colors 1 natural color 0 natural colors	2 1 0
VIII	Odors – a high intensity of positive smells – high intensity of negative odors – moderate intensity of positive smells – moderate intensity of negative odors – low intensity of positive smells – low intensity of negative odors	10 –10 5 –5 1 –1
IX	Aeration – high – moderate – low	3 2 1
X	Perceived humidity – high – moderate – low	3 2 1
XI	Perceived noise – severe – moderate – low	0 5 10

This combined method created a substantial basis for the implementation of this field research. The study area was determined by applying 50 x 50 m squares (black line, Figure 2) onto the map of the area analyzed at a scale of 1:5 000. Then we distinguished squares with a 100 m side (yellow line, Figure 2), which reflected the route frequently traveled by visitors. The applied two-step breakdown enabled the best possible and most accurate selection of representative areas. The analyzed area included thirty-seven 100 x 100 m squares.

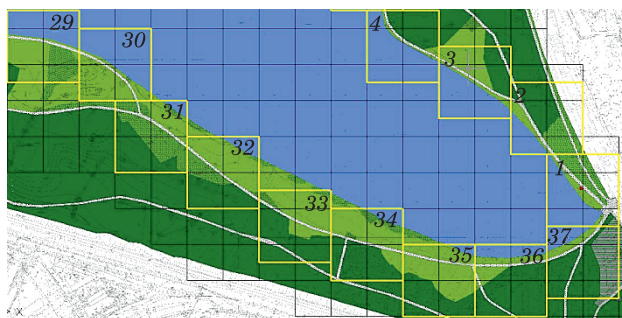


Fig. 2. A two-stage division of land into a grid of squares with their numbers

The study commenced in square number 1 (Figure 2) and continued in a north-western direction around the lake. The research was carried out between June and September 2012. The multisensory valorization of the landscape took place in the center of the designated 100 00 m squares and included a 180° field of view. It should be noted that the assumed barrier introduced a limit for the senses other than sight. Odors, light, noise, aeration and humidity were assessed at the central point of the determined area.

It was noted that sensations associated with sight could not be strictly divided into predetermined distances. It can be argued that the assumed distances in the applied division of space are misleading as the evaluation of a landscape usually involves the entire view up to the horizon perceived as a whole. Determination of artificial barriers limits the scope of assessment and ignores this overall view.

## **Results and Discussion**

Lake Rusalka is a popular recreation site not only in summer but also in spring and autumn. The forest area adjacent to the lake has a popular bike trail. Designated walking paths, partly paved with rubble and asphalt, are used by cyclists, walkers and horse riders. The area is also attractive to anglers, although night angling and spinning are prohibited. In the middle of the northern shore are the facilities of the local center of sport and recreation (POSiR), which takes care of a patrolled swimming area equipped with a pier, slide into the water, changing rooms and dining facilities.

Our inventory shows that both the buildings and general infrastructure are in poor condition. There is also a high number non-official shortcuts across green areas, which indicates an improper arrangement of paths for pedestrians and cyclists. The number of bins is sufficient, although their size, color and distribution should be changed. All these defects lower the value of the area, and the current status results in a growing discontent among visitors.

The picturesque landscape of Lake Rusalka can be truly appreciated and not affected by negative sensations after leaving the area belonging to POSiR. The landscape includes a smooth transition of lawns going down to the water. A large number of trees that surround the area provide a chance to rest in the shade during periods of intense sunlight. In autumn the scenery of the lake becomes even more attractive. The nearby forests are dominated by deciduous species, distinguished by leaves assuming various colors in autumn with various shades of yellow, red and brown. Around the lake one can see a large number of visitors who choose this side of the lake not occupied by buildings and is not significantly transformed by man.

Perhaps contact with nature and the real chance to relax in a smaller group of fellow visitors is the most important aspect of the area for people (MOON et al. 2006). The area is a good place for active recreation and family walks, and enables close contact with nature which is especially valuable for residents of large cities.

At the initial stage of the evaluation, the type of landscape was defined as a surface water landscape with a deep horizon, partly or completely covered. It can be characterized as a picturesque landscape, where on a clear day the sunlight glistens on the water.

The surrounding vegetation provides shelter from excessive sunlight. In this area, the predominant colors are blue and green, which can be invigorating and calming for city dwellers. During a sunny day, the temperature conditions are favorable, while at night and in the morning the area has a lower temperature and considerable humidity. These are conditions conducive to noise propagation, which may become irritating after a longer visit.

After analyzing all the elements included in the aforementioned multisensory method of landscape valorization for each study area, the obtained point results were divided into 3 intervals with distinct differences in scores. This indicates a considerable variety in the study areas and also a wide range of bonitation points adopted in the method.

Designation of individual zones around the lake was performed only after a comprehensive valorization of the entire area. Those plots which obtained the lowest scores (16–30) are marked with a red line (Figure 3). Low scores

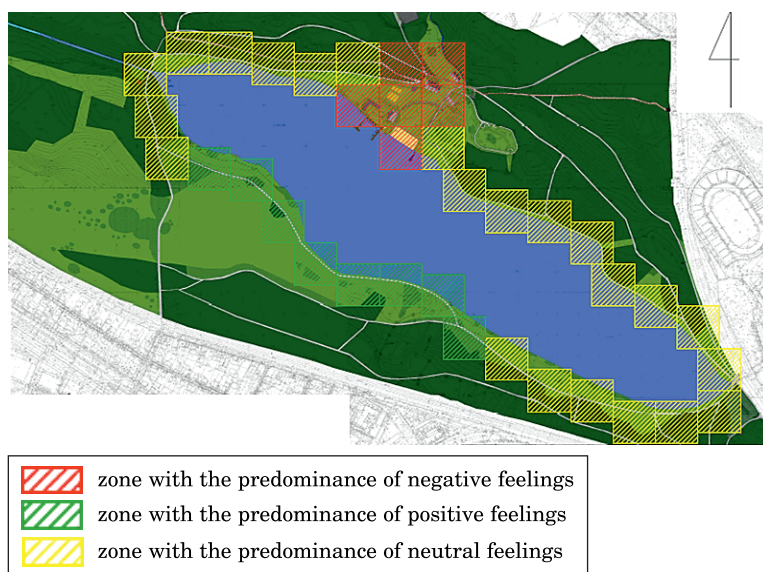


Fig. 3. Multisensory landscape valorization – zoning



indicate a need for immediate revitalization, and were due mainly to negative feelings resulting from the low aesthetic value and the sense of disharmony (Figure 4a). Another significant element was the high intensity of unpleasant odors from the nearby fish restaurants. A yellow line corresponds to areas which received moderate scores (ranging from 37 to 52). These areas were marked by a lack of significant negative or positive elements affecting the landscape (Figure 4b). Landscape in these areas can be considered neutral for observers. The areas with the highest point scores (ranging from 57 to 59) are indicated by the green line. The high score was related to sites that induced positive feelings. Aesthetics and harmony in these areas were not perceived as distorted and had a positive impact on the recipient (Figure 4c).

*a**b**c*

Fig. 4. Landscape of Lake Rusalka: *a* – the worst zone, *b* – medium zone and *c* – the best zone  
Phot. K. Tomasz

The use of this simple technique was meant to establish individual zones that require revitalization. Table 2 shows the total results for each of the squares.

Table 2

The aggregate value of multisensory landscape valorization together with the designation of zones

Numbers of the squares	Number of points	Zone	Numbers of the squares	Number of points	Zone	Numbers of the squares	Number of points	Zone
1	42	neutral feelings	16	41	neutral feelings	32	48	neutral feelings
2	47		17	42		33	46	
3	52		18	40		34	47	
4	52		19	37		35	46	
5	52		20	43		36	46	
6	52		21	49		37	40	
7	50		22	49		32	48	
8	47		23	46				
9	44		24	57	positive feelings			
10	18	negative feelings	25	58				
11	16		26	59				
12	30		27	59				
13	26		28	57				
14	26		29	57				
15	26		30	58				
			31	58				

After selecting the zones (Figure 3) it turned out that the land belonging to POSiR was the area in the greatest need of revitalization. The best zone was located at the opposite bank of the lake. Its green meadows have a great potential, while rather unpleasantly looking infrastructure on the northern shore created, paradoxically, a pleasant panorama when seen from a distance, and had a positive impact on aesthetic feelings. The sense of harmony in the area was not perceived as disrupted.

The best and the worst zones were buffered by an intermediate zone, an area in need of only low-scale remedial treatment.

The land belonging to POSiR can be perceived as the most neglected and accordingly received a low score. On the other hand, after corrective management it can become the best area combining human activity and natural beauty. It can create an ideal place for recreation for the whole family. Lake Rusalka is a universal site where one can spend free time at any time of the year. This feature should be emphasized by proper management.

Adequate planning and technical development should enable correct development of the zone, attract tourists and allow extension of the duration of the season and increase the tourism potential of the Lake (KURLETO 2013,

REMENYIK et al. 2013, RODRIGUES et al. 2013). Appropriate solutions should highlight the potential of the area and could become its great assets, as evidenced by examples of successful landscape revitalization projects all over the world (BRUTTOMESSO 2001, MAJDECKA-STRZEŻEK 2009).

The method of evaluation in this study is an attempt at the most accurate determination of factors that influence the perception of a landscape by observers. It defines and identifies the most important stimuli that affect perception of the surrounding landscape, making it possible to reconcile different concepts of landscape perception (KOWALCZYK 1992, KAPLAN et al. 2006, BERNAT 2012). Adding other senses to visual perception makes the method more comprehensive than traditional methods, and enables the processing of the observer's feelings into a clear scale of points (PORTEOUS 1985, PORTEOUS and MASTIN 1985, SCHAFER 1994).

## Conclusions

The developed method of multisensory landscape valorization enables the conversion of the observer's feelings into a clear point scale. Because sensory (and elusive) impressions are presented with the use of numbers, the results inform about the needs of designated areas in a clear and understandable manner. This allows a maximally objective distinction of zones and identification of their needs in terms of revitalization. The used multisensory method of landscape valorization has successfully designated the locations most urgently requiring value.

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## NOSEMOSIS IN HONEY BEES

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**Key words:** Microsporidia, *Nosema apis*, *Nosema ceranae*, *Apis mellifera*, *Nosemosis*.

### Abstract

Microsporidia (*phylum Microsporidia*) are single-celled eukaryotic organisms and obligate intercellular parasites that produce spores. They are classified under the *Fungi* kingdom. In honey bees (*Apis mellifera*), microsporidian infections are caused by *Nosema apis* and *Nosema ceranae*. Bees are infected per os by food contaminated with spores. Spores were observed in intestinal epithelial cells, the Malpighian tubule system, salivary glands and fat bodies. Nosemosis symptoms include digestive and absorption disorders because spores damage epithelial tissue of the alimentary canal that is responsible for food absorption. *Nosema* spp. spores are routinely determined under a light microscope, and they are identified to species level by PCR with the use of 16S rRNA primers designed for small subunits. Nosemosis treatments are regulated by European Union directives and recommendations of the World Health Organization.

## NOSEMOZA PSZCZÓŁ

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**Słowa kluczowe:** Microsporidia, *Nosema apis*, *Nosema ceranae*, *Apis mellifera*, *Nosemosis*.

### Abstrakt

Microsporidia (gromada *Microsporidia*) należą do eukariotycznych organizmów jednokomórkowych, które są obligatoryjnymi wewnątrzkomórkowymi pasożytami wytwarzającymi spory. Zaliczane są do grzybów (*Fungi*). Przedstawicielem Mikrosporidii występujących u pszczoły miodnej (*Apis mellifera*) jest *Nosema apis* i *Nosema ceranae*. Do zarażenia dochodzi drogą *per os* po spożyciu zakażonego pokarmu. Stwierdzono występowanie spor w komórkach nabłonka jelit, w cewkach

Malpighiego, gruczołach ślinowych, ciele tłuszczowym. Objawy nosemozy związane są z zaburzonymi procesami trawienia i przyswajania pokarmu, ponieważ *Nosema* uszkadza nabłonek w przewodzie pokarmowym. Do rutynowego rozpoznania sporocyst *Nosema* spp. używa się mikroskopu świetlnego, a przynależność gatunkową potwierdza się za pomocą metod PCR, wykorzystując startery zaprojektowane dla małej podjednostki 16S rRNA. Leczenie nosemozy jest objęte dyrektywami Unii Europejskiej oraz zaleceniami Międzynarodowej Organizacji Zdrowia.

Recent years witnessed an increase in bee mortality in many regions of the world. The drop in bee populations is associated with viral, fungal and parasitic diseases, pesticide poisoning, monoculture farming and pollen shortage. The direct cause of the colony collapse disorder (CCD), a phenomenon in which entire colonies abruptly disappear from a beehive without an apparent reason (HIGES et al 2008b, 2009), has not been identified to date. Similar changes in bee colonies had been reported earlier, but scientists were unable to find any links between those processes (HIGES et al. 2006, PORRINI et al. 2010). CCD is caused by a combination of many factors (COX-FOXTER 2007). One of the main causes of the syndrome are spores of the genus *Nosema* spp. (PAXTON 2010, CHAIMANEE et al. 2010), including *N. apis* and *N. ceranae* fungi (kingdom *Fungi*, phylum *Microsporidia*, family *Nosematidae*, genus *Nosema*). The above parasites cause nosemosis (*Nosemosis apium*), a microsporidian infection that affects adult bees (SNEATH and SOKAL 1973, FRIES 2010).

Microsporidia (class *Microsporea*) are single-celled eukaryotic organisms and obligate intercellular parasites that produce spores. They colonize both vertebrates and invertebrates, and their spores are characterized by the presence of polar fibers (GRACZYK et al. 2007). Microsporidia are widely distributed in nature, and they comprise more than 1200 species, of which at least 14 are dangerous for humans. Patients with impaired immune function, in particular HIV carriers, and organ transplant patients are particularly susceptible to microsporidian infections (DONG et al. 2010, GRACZYK et al. 2007).

The first microsporidian species of *Nosema bombycis* was discovered in the silkworm (*Bombyx mori*) and described by Nageli in 1857 (NAGELI 1857). In the same year, Donhoff performed a microscopic analysis of small formations isolated from the intestines of adult bees and identified them as fungi. Pasteur discovered many infectious diseases caused by pathogens, including *N. bombycis*, in silkworms. In 1909, Zander identified the spores present in bee intestines as parasites and named them *Nosema apis* (ZANDER 1909). It is generally believed that Danhoff and Zander identified the same parasite. *N. bombycis* is transmitted transovarially in 100%, whereas other *Nosema* species – in only 1.2% (HAN and WATANABE 1988) – Table 1.



Table 1

The presence of *Nosema* spp. species of insects

Host	Species <i>Nosema</i>
Mosquito ( <i>Culex</i> spp.)	<i>N. algerae</i>
Mullberry silkworm ( <i>Bombyx mori</i> )	<i>N. bombycis</i>
Honey bee ( <i>Apis mellifera</i> )	<i>N. apis</i> <i>N. ceranae</i>
Locust ( <i>Locusta migratoria</i> )	<i>N. locustae</i> <i>N. grylli</i>
Fly ( <i>Drosophila melanogaster</i> )	<i>N. kingii</i>
Chrysolina beetle ( <i>Chrysolina</i> spp.)	<i>N. coulloudi</i>
Wasp ( <i>Vespula</i> )	<i>N. vepsula</i>
Moth ( <i>Heterocera</i> spp.)	<i>N. lymantriae</i> <i>N. serbica</i>

In honey bees (*Apis mellifera*), microsporidian infections are caused by *Nosema apis* and *Nosema ceranae*. Honey bees initially colonized only Africa, the Near East and Europe. They were introduced to America, Australia and Asia by colonizers those regions. Today, honey bees inhabit geographically diverse areas, and the species of *A. mellifera* includes various African, Oriental and European breeds. Numerous breeding lines have been engineered by humans, in particular in European breeds (TOMASZEWSKA and CHORBIŃSKI 2000). *Nosema* infections were observed in the following bee species: *Apis mellifera*, *A. ceranae*, *A. florea* and *A. dorsata* (CHAIMANEE et al. 2010).

### Development of *Nosema* spores in bees

Bees are infected orally by food contaminated with spores (CHEN et al. 2008, WEBSTER et al. 2004). The optimal temperature for spore growth in bee intestines is 30–34°C, and spores remain active for more than seven months. In bees, the spread of nosemosis is determined mainly by weather conditions during various seasons of the year. Nosemosis caused by *N. apis* develops on a seasonal basis, and the highest prevalence of the disease is noted in spring when bee populations increase. The pathogenic process is stabilized in summer when infection levels are low. A repeated increase in pathogen counts is noted in fall (GAJDA 2010, HIGES et al. 2006). Recent research indicates that *N. ceranae* infections are more prevalent in *A. mellifera* than infections caused by *N. apis*. Bees infected by *N. ceranae* quickly die, usually outside the hive, without displaying any clinical symptoms (CHEN et al. 2009, FORSGREN and FRIES 2010, HIGES et al. 2007, PAXTON et al. 2007). The prevalence

of *N. ceranae* infections remains similar throughout the honey season, which is the main distinguishing feature from infections caused by *N. apis* (KLEE et al. 2007, MARTIN-HERNANDEZ et al. 2007). The discussed parasites also differ in the length of their developmental cycle, which has been determined at five days in *N. apis* and three days in *N. ceranae*. Intestinal epithelial cells become infected already three days after the parasitic attack, and the insect usually dies within nine days, especially in the presence of other stressors such as bacteria or viruses. The analyzed pathogens were identified not only in intestinal epithelial cells, but also in the Malpighian tubule system, salivary glands and fat bodies (CHEN and HUANG 2010).

The developmental cycle of *Nosema* spp. takes place in several stages. The spore ejects a polar filament upon entering the insect's middle intestine. A planont, a motile amoeboid form of the parasite measuring approximately 1  $\mu$ , emerges from the capsule. Initially, the planont has two nuclei that are later merged. The planont penetrates mid-intestinal epithelial tissue where it feeds and loses motility. The fungus grows and begins to divide. Multiplying cells are known as meronts. Meronts have the size of 3.3–7.5  $\mu$ . Multiplying meronts fill the entire epithelial cell, destroy its protoplasm and, in some cases, damage the nucleus. In an unsupportive environment, meronts cease to multiply and turn into spores. This transformation takes place in three ways. In the first case, the meront nucleus becomes elongated and narrow, and it divides to produce two daughter cells. Each daughter cell gives rise to a sporoblast that is transformed into a spore. In the second case, the meront nucleus becomes elongated and undergoes multiple division. This process gives rise to multinucleate meronts, and the number of produced spores is equal to the number of nuclei. In the third case, the meront preserves its round shape, the nucleus undergoes multiple division to create multinucleate plasmodium. The number of spores formed inside the plasmodium is equal to the number of nuclei. Unlike meront nuclei, sporont and sporoblast (intermediate forms between a meront and a spore) nuclei do not have a protective capsule, and they comprise numerous separate granules grouped in clusters. Developing spores fill the entire mid-intestinal epithelial cell that eventually dies and exfoliates into the intestinal lumen where it disintegrates or is evacuated with other spores (DIDIER et al. 2000) – Figure 1.

The symptoms of *N. apis* infections include digestive and absorption disorders because spores damage epithelial cells of the alimentary canal that is responsible for food absorption. Infected bees excrete large amounts of sweet watery stool (undigested food). The feces of diseased individuals contain large numbers of spores, and they are the main source of infection. Infected feces are excreted by bees during flights to the apiary and water sources, and in unfavorable weather conditions, also inside the hive – on honeycombs, honey

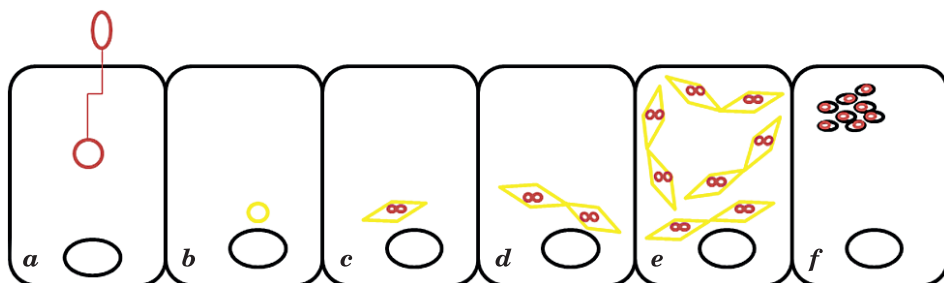


Fig. 1. Development scheme *N. apis* i *N. ceranae* in the midgut epithelial cells of bees: *a* – penetration of polar spores into the host cell; *b* – sporoplasm (round-shaped) in the epithelial cell; *c* – development meronts; *d* – division and the formation meronts; *e* – division meronts; *f* – formation oval sporonts

supers, frame bars, walls and the bottom board. In most cases, the disease spreads when healthy colonies are merged with infected colonies, and when contaminated hive equipment and food reserves are used. Infections are potentiated by stressors, such as the loss of the bee queen, changes in the hive microclimate and the presence of weak colonies that are unable to accumulate the required food reserves, mainly pollen. Symptoms of disease include swollen abdomen and grayish-white discoloration of the middle intestine.

Four stages of *N. ceranae* infection have been identified in *A. mellifera* bees (HIGES et al. 2008). The first stage is asymptomatic, and it lasts from spring to early fall. There are no discernible changes in the size of hive colonies or broods. The second stage is colony replacement, and it is observed between late fall and winter. Bees begin to die when the temperature drops (energy stress), and the queen attempts to make up for that loss by laying more eggs. The size of the bee population remains unchanged, but the brood increases. The queen begin to lays eggs in winter, which is often mistakenly interpreted as a sign of colony health. The third stage is false recovery when hive populations are high and all frames are filled with brood. Despite the large size of colonies, bees do not swarm. The last stage is depopulation, namely the sudden collapse of entire colonies. A small number of bees, the queen and infrequent brood survive the infection. Substantial food reserves are accumulated in the hive. The depopulation stage is observed mainly in fall or early winter. Less virulent infections may lead to colony collapse in spring.

*Nosema* spp. spores are routinely determined under a light microscope. Analyses are generally performed in early spring on samples collected from winter hive debris (mostly worker bees) (TOPOLSKA and KASPRZAK 2007, MICHALCZYK et al. 2011). *Nosema* spp. parasites are difficult to identify under a light microscope due to minor differences in the anatomy of *N. apis* and

*N. ceranae* spores. *N. ceranae* spores have the length of 3.3–5.5 µm and the width of 2.3–3.0 µm. *N. apis* spores are larger with the length of 4.6–6.4 µm and the width of 2.5–3.4 µm, they have a regular, cylindrical shape with one slightly tapering end, and they strongly refract light under a light microscope (FRIES et al. 2006). *N. apis* and *N. ceranae* spores have a similar morphological structure, and the main difference that can be observed under an electron microscope is the length of the polar filament (FORSGREN and FRIES 2010). For this reason, spores are identified to species level by PCR with the use of 16S rRNA primers designed for small subunits (HIGES et al. 2006, KASPRZAK and TOPOLSKA 2007). The presence of spores in a bee colony can also be determined in a field test that involves the preparation of mid-intestinal specimens and observations of their color. Healthy bee intestines are yellow to brown in color, whereas infected intestines turn white.

In nurse bees, microsporidian infections inhibit the development of pharyngeal glands that secrete royal jelly, which could disrupt the feeding patterns of queen bees and the brood (GLIŃSKI and RZEDZICKI 1993). *N. apis* infections shorten the average life of worker bees by 20–50% and of queen bees by 30–75%, they lower honey production by 60% and wax production by 25%. In diseased colonies, the brood can be reduced by even 50%, and highly virulent infections may lead to ovarian damage and infertility in queen bees (WEBSTER et al 2004, SAGASTUME et al. 2011).

*N. ceranae* infections develop rapidly and are highly lethal. Bees die within 8 days after exposure to the pathogen (HIGES et al. 2007). Recent research demonstrated that *N. ceranae* had developed more effective mechanisms of adaptation to changing temperatures than *N. apis*. At temperatures that limit fungal development (25 and 37°C), *N. ceranae* is able to complete its lifecycle, whereas the lifecycle of *N. apis* is inhibited. At the optimal temperature of 33°C, *N. ceranae* is able to destroy 2–3 times more intestinal epithelial cells than *N. apis* (FENOY et al. 2009). Infections caused by *N. ceranae* in bee colonies last one year and can remain asymptotic, whereas the disease spread by *N. apis* disappears in warm months of the year, often at the beginning of the honey season. *N. ceranae* infections do not produce diarrhea, which always accompanies *N. apis* infections, and they are often referred to as „dry” nosemosis (FAUCON 2005, MAYACK and NAUG 2009). In Poland, worker bees are more frequently infected with *N. ceranae* than *N. apis* spores. In a multiplex PCR analysis of 1000 winter hive debris samples, the presence of *Nosema* spp. DNA was found in 806 samples (80.6%), including *N. ceranae* in 206 samples (20.6%), mixed infections (*N. apis* / *N. ceranae*) were noted in 600 samples (60%), 194 samples (19.4%) were free of the analyzed pathogens, and none of the examined samples were infected by *N. apis* only (MICHALCZYK et al. 2011).

Nosemosis treatments are regulated by European Union directives and recommendations of the World Health Organization. The use of pharmacological products such as Fumagilin DCH and Fumidil B is discouraged because their residues may contaminate bee products. Sanitary and preventive methods are recommended to minimize the risk of disease, including stimulating flight activity in early spring, providing hives with adequate sunlight exposure and positioning hives at a distance to prevent bees from entering the wrong hive. In spring, hive debris should be removed to prevent bees from coming into contact with infected individuals in the bottom board. Hive walls and frames contaminated with feces also contribute to the spread of disease, and they should be chemically disinfected. In early spring, healthy bees can be fed water syrup with pollen to stimulate their alimentary canal, and diseased bees should be administered dietary supplements, such as Api-Herb, Nozevit, Vita Feed Gold, Protofil and Noestat. Treatment of nosemosis is difficult and often ineffective. Continuous efforts are being made to identify new substances that effectively treat *Nosema* infections and are safe for bees and consumers of bee products.

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