ACTA UNIVERSITATIS CAROLINAE

European Journal of Environmental Sciences

VOLUME 3 / NUMBER 1
2013

CHARLES UNIVERSITY IN PRAGUE
KAROLINUM PRESS
2013

ISSN 1805-0174

CONTENTS

TEMPORAL AND SPATIAL CHANGES IN THE CONCENTRATIONS OF RADIOCAESIUM AND RADIOSTRONTIUM IN THE VLTAVA RIVER BASIN AFFECTED BY THE OPERATION OF THE NUCLEAR POWER PLANT AT TEMELÍN

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ABSTRACT

Concentrations of radiocaesium and radiostrontium were assessed in surface water, sediment, fish and aquatic flora affected and not affected by waste water discharges from the Temelín Nuclear Power Plant. The assessment included residual contamination originating from nuclear weapon tests in the atmosphere over the last century and the accident at Chernobyl in 1986. Results of long-term monitoring (since 1990) were used to derive effective ecological half-lives of these radionuclides in the hydrosphere. Possible effect of waste water discharged from Temelín power plant on the concentrations of radiocaesium and radiostrontium in the Vltava River was assessed by using data recorded in the period 2001–2010.

Keywords: nuclear power plants, radiocaesium, radiostrontium, hydrosphere

Introduction

This paper focuses on an analysis of the results of long-term monitoring and assessment of concentrations of radionuclides in the hydrosphere in the vicinity of the Nuclear Power Plant at Temelín.

This nuclear power plant discharges waste water that contains radioactive substances, of which tritium is the most important. The waste water also includes other radionuclides, which originate mainly from local surface water used to cool the reactor in the plant along with minor quantities that originate from the standard operation of the plant. The radionuclides that could have originated from the power plant include mainly tritium, as the amounts of fission products, such as radiocaesium and radiostrontium, released during routine operation are very low. Average annual amount of ^{137}Cs and ^{90}Sr released from the Temelín plant in the period 2002–2010 was 0.057 GBq y^{-1} and 0.001 MBq y^{-1}, respectively (Hanslík 2000–2011). The radionuclides of artificial origin were detected in the environment in the vicinity of the Temelín plant before it started operating in 2001 and attributed to radionuclide contamination of the environment that resulted from tests of nuclear weapons in the atmosphere in the fifties and sixties in the 20th century and the accident at Chernobyl in 1986.

Caesium 134 and 137 (^{134}Cs, ^{137}Cs) and strontium 90 (^{90}Sr) are radioecologically important radionuclides, which remain in the environment for a long time because they have long half-lives (2.1, 30.2 and 28.8 y). In previous research projects it was mainly the concentration of ^{137}Cs that was measured because the concentrations of this radionuclide can be determined using gammaspectrometric analysis, which requires special equipment, but its determination is less time consuming than the radiochemical determination of beta emitters such as ^{90}Sr. This accounts for the little data and knowledge of the concentrations of ^{90}Sr compared to other radionuclides in the Czech Republic and elsewhere (Outola et al. 2009).

Construction and operation of the Temelín plant initiated the implementation of a number of projects, which focused on the possible effects of this power plant on the environment. This resulted in the collection of data, which currently spans a period of 20 years. The first two projects (Hanslík 1995; Hanslík 1998) were sponsored by the Czech Ministry of the Environment and their main objectives were to determine the pre-operational environmental conditions (a reference level) in terms of concentrations of radioactive and non-radioactive substances present in the environment, particularly in the hydrosphere, and predict possible effects of the future operation of the Temelín power plant. This monitoring and research continued during the period 1999–2010 within the framework of a project sponsored by the Czech Power Company (Hanslík 2000–2011) and one on the Research and protection of the hydrosphere – research on the relationships and processes in the water component of the environment, which focused on effects of human pressure, the sustainable use and protection of the hydrosphere and legislative tools (MZP 0002071101 sponsored by the Czech Ministry of the Environment). The main objectives of these projects were to assess the effects of the waste water discharged from the Temelín power plant on the concentration of tritium, radiocaesium and radiostrontium in the hydrosphere, compare these effects with the residual contamination from the tests of nuclear weapons in the atmosphere and the Cher-

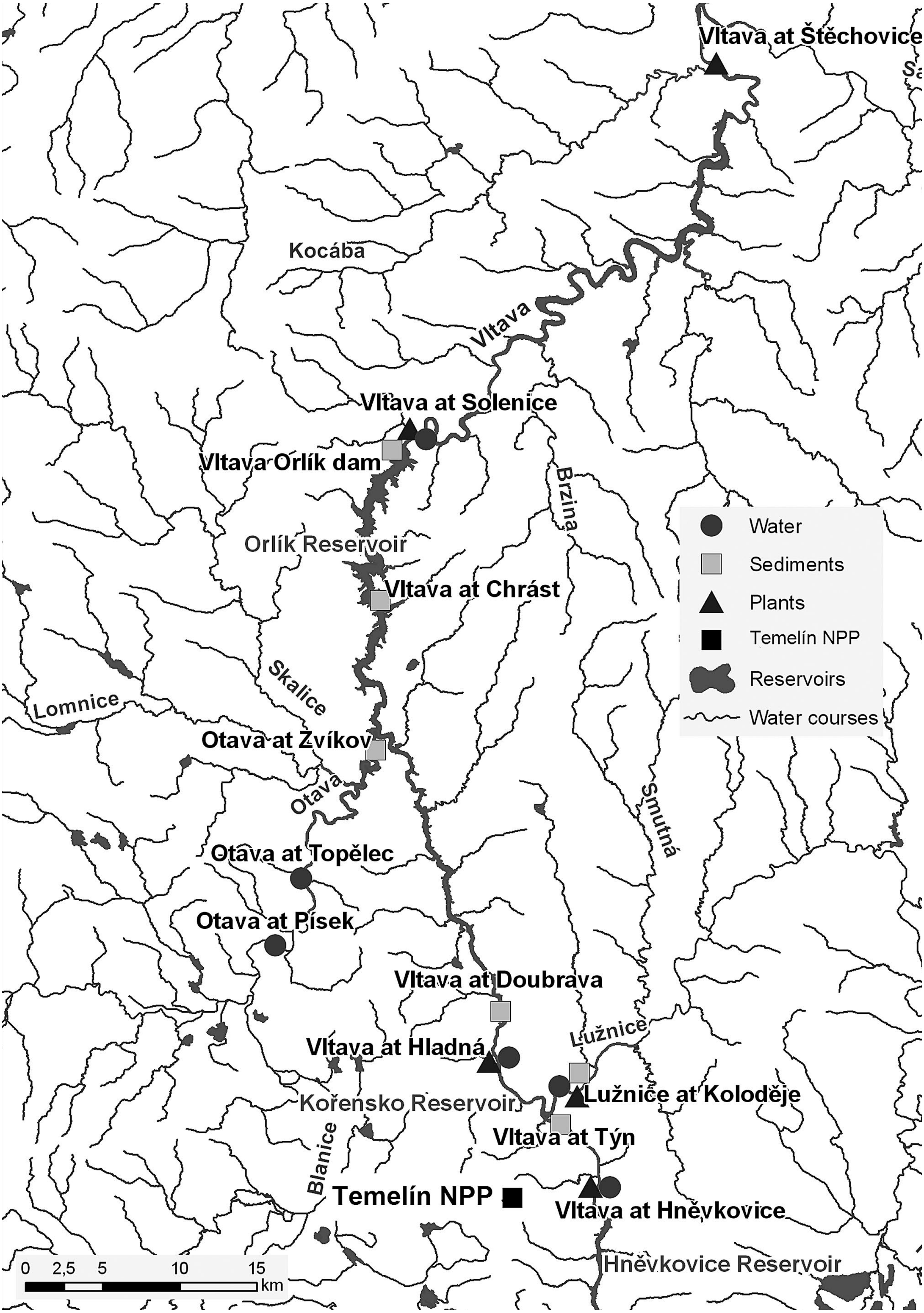

Fig. 1 Map of sampling sites.

nobyl accident in the last century and evaluate the long-term spatial and temporal trends in the concentrations of these radionuclides monitored at sites not affected and affected by the outflow of waste water from the Temelín power plant. This paper reviews and summarizes the main results of these projects in terms of radiocaesium and radiostrontium. The results on tritium were previously published by Ivanovová and Hanslík (2010).

Methods

The changes in the concentrations of radiocaesium and radiostrontium were monitored in the tributaries of the Orlík Reservoir, the Vltava, Lužnice and Otava Rivers, which are located upstream from the waste water outflow from the Temelín power plant and represent the reference conditions ("reference sampling sites"), and in the Vltava River downstream from the outflow of waste water from the power plant ("affected sampling sites"). The monitoring involved determining concentrations in surface water, bottom sediments, fish and water plants (Fig. 1). The monitoring of the concentrations in the surface water and sediments was started in 1990, fish during the period 1986–1990, in 1994 and annually since 1998, and aquatic plants in the period 1996–2010.

The collection and processing of the samples follow those specified in ČSN EN 25667-1 (1994) and 2 (1994) Standards and ČSN EN ISO 5667-3 (1996), 4 (1994) and 6 (1994). Quality control practices of the T.G.M WRI Radioecological Laboratory were followed as specified in the Standard ČSN EN ISO/IEC 17025 (2001; 2005). This laboratory is responsible for the national and international proficiency testing.

Four separate large samples of water (50 l) were collected each year. These samples were immediately stabilized with nitric acid to pH 1 and then, after transportation to the laboratory, dried by vaporization. The vaporized samples were ignited (350 °C) and enclosed into Petri dishes. The radionuclides concentrations in water were determined in total solids (both in dissolved and suspended solids). Samples of water (1 l) for determining total suspended solids (TSS) were collected with the same frequency. Samples of sediment were collected by a diver from the top layer (0–10 cm) of the sediment at the bottom at six sites in tributaries of the Vltava River and Orlík Reservoir. The frequency of sampling was once a year. Granularity of the samples was generally less than 2 mm. For the analysis, the samples were dried at 105 °C and hermetically sealed in measuring containers. Samples of fish and water plants were also collected annually. These samples were dried at 105 °C and hermetically sealed in measuring containers.

The ^{134}Cs and ^{137}Cs concentrations were analyzed following the Standard ČSN ISO 10 703 procedure (1999; 2008) using gamma-spectrometry. A Canberra device was used. The measurement duration was set to the requested minimum detectable activity (MDA) of ^{137}Cs and level of significance of $\alpha = \beta = 0.05$. The MDA of ^{137}Cs in water counted over a period of 2 days was 0.5 mBq l^{-1}. In the sediments, the MDA of ^{137}Cs counted over a period of 8 h was approximately 0.5 Bq kg^{-1}. The results recorded for ^{137}Cs activity in fish (dried samples) were converted to activity in terms of wet weight. The MDA of ^{137}Cs in fish (wet weight) counted over a period of 2 days was 0.1 Bq kg^{-1}. ^{90}Sr in water was determined by using a standard method after radiochemical separation (Hanslík 1993). Its activity was detected in the residue left after ignition and detected using yttrium 90 after radiochemical separation. Value of MDA of ^{90}Sr was 3 lmBq l^{-1}. These methods were verified and recommended by the International Atomic Energy Agency (IAEA 1996) in Vienna as part of the technical assistance agreement with the Ministry of the Environment of the CR and the State Office for Nuclear Safety.

The data on the concentrations of radionuclides in water, sediments and biomass were assessed using several mathematical methods, which are briefly described below.

The effective ecological half-lives were determined using the decrease in the activity of a radionuclide and the equation published by Smith and Beresford (2005):

$$T_{eff} = \frac{\ln 2}{\lambda_{eff}} \tag{1}$$

where T_{eff} is effective ecological half-life (y) and λ_{eff} is effective ecological decay constant of the radionuclide activity concentration (y^{-1}).

Ecological half-lives were calculated by using the equation proposed by Smith and Beresford (2005):

$$\frac{1}{T_{ecol}} = \frac{1}{T_{eff}} - \frac{1}{T_P} \tag{2}$$

where T_{ecol} is ecological half-life (y) and T_p is physical half-life (y).

For trend analyses a kinetic equation of the first order of the following form was used (an example for ^{137}Cs):

$$\ln(c_{137\mathrm{Cs},j}) = -\lambda_{eff} \cdot t + q \tag{3}$$

where $c_{137\mathrm{Cs},j}$ is annual average concentration of ^{137}Cs in surface water (Bq m^{-3}) in year j, λ_{eff} is effective rate of decline in concentration of ^{137}Cs (y^{-1}), involving the physical decay constant (λ_p) and ecological rate of decrease (λ_{ecol}), $\lambda_{eff} = \lambda_p + \lambda_{ecol}$ (y^{-1}), t is incidence of the monitoring measured in years, and q is natural logarithm of activity at the beginning of the observation.

The ecological and effective ecological half-lives were calculated for a confidence level of 68% using the ERA 3.0 program.

The annual depositions of suspended solids in a reservoir were calculated using the following equation:

$$D_{\mathrm{S,j}} = \left(\sum_{t=1}^{n} c_{S,j,t} \cdot Q_{j,t} + c_{S,j,ia} \cdot Q_{j,ia} - c_{S,j,o} \cdot Q_{j,o} \right) \cdot t \cdot 10^{-3} \tag{4}$$

where $D_{S,j}$ is deposition of suspended solids in a reservoir in individual years (j) (t y^{-1}), $c_{S,j,t}$ is mean annual concentration of suspended solids (j) in individual tributaries (t) (kg m^{-3}), $c_{S,j,ia}$ is mean annual concentration of suspended solids (j) in the inflow from inter-basin area (ia) (kg m^{-3}), $c_{S,j,o}$ is mean annual concentration of suspended solids (j) in the outflow from a reservoir (kg m^{-3}), $Q_{j,t}$ is mean annual inflow (j) from individual tributaries (t) (m^3 s^{-1}), $Q_{j,ia}$ is mean annual inflow (j) from the inter-basin area (m^3 s^{-1}), and $Q_{j,o}$ is mean annual outflow (j) from a reservoir (m^3 s^{-1}).

The deposition of suspended solids was also calculated for individual years using the following formula and expressed in percentages:

$$D_{S,j} = \frac{\sum_{t=1}^{n} c_{S,j,t} \cdot Q_{j,t} + c_{S,j,ia} \cdot Q_{j,ia} - c_{S,j,o} \cdot Q_{j,o}}{\sum_{t=1}^{n} c_{S,j,t} \cdot Q_{j,t} + c_{S,j,ia} \cdot Q_{j,ia}} \cdot 100 \tag{5}$$

where $D_{S,j}$ is the deposition of suspended solids in a reservoir in individual years (j) (%).

Similarly, based on the results of monitoring the ^{137}Cs activity in all substances in water (dissolved as well as undissolved solids), the deposition of ^{137}Cs can be determined using the formula:

$$D_{A,137Cs,j} = \left(\sum_{t=1}^{n} c_{137Cs,j,t} \cdot Q_{j,t} + c_{137Cs,j,ia} \cdot Q_{j,ia} - c_{137Cs,j,o} \cdot Q_{j,o} \right) \cdot t \cdot 10^{-9} \tag{6}$$

where $D_{A,137Cs,j}$ is the deposition of ^{137}Cs in individual years (j) (GBq y^{-1}), $c_{137Cs,j,t}$ is mean annual activity of ^{137}Cs (j) in individual tributaries (t) of a reservoir (Bq m^{-3}), $c_{137Cs,j,ia}$ is mean annual activity of ^{137}Cs (j) the inflow from inter-basin area (ia) (Bq m^{-3}), $c_{137Cs,j,o}$ is mean annual activity of ^{137}Cs (j) in the outflow from a reservoir in Bq m^{-3}.

Concentration factors (CF) for fish samples were calculated using the equation recommended by Smith and Beresford (2005):

$$CF = \frac{\bar{a}_{137Cs}}{\bar{c}_{137Cs}} \tag{7}$$

where is annual weighted average concentration of ^{137}Cs in fish (wet weight) (Bq kg^{-1}), and is annual average concentration of ^{137}Cs in water in tributaries of Orlík Reservoir (Bq l^{-1}).

The concentration factors were calculated for a confidence level of 68% as standard deviations.

Results and discussion

Concentrations of ^{137}Cs and ^{90}Sr in water

Temporal changes in the concentrations of ^{137}Cs in water samples collected from Orlík Reservoir and its tributaries in two periods, 1990–1994 and 1995–2010, were determined. The effective ecological half-lives (T_{eff}) in water in individual tributaries and the outflow from Orlík Reservoir (Table 1) were in the range of 1.1–2.2 y for the period 1990–1994 and 6.2–10.9 y for the period 1995–2010. The ecological half-lives (T_{ecol}) were in the range 1.2–2.4 y for the period 1990–1994 and 5.1–8.0 y for the period 1995–2010. The results of these studies showed that the decrease in the concentrations of ^{137}Cs, which was observed before the power plant began operating, continued also during the subsequent period. An example is shown in Fig. 2 for the Vltava River at Hněvkovice (a reference sampling site, source of technological water) and Solenice (downstream from the Temelín waste water outflow). In 2010, the average activity of ^{137}Cs at Hněvkovice was 0.5 mBq l^{-1} and at Solenice 0.3 mBq l^{-1}.

The results of these studies carried out in the vicinity of the Temelín plant are in agreement with those of similar studies on changes in water contamination after the Chernobyl accident. For example, Zibold et al. (2001) recorded a fast decrease in the concentration of ^{137}Cs in the period 1986–1988 and a second slower decrease

Table 1 The evaluated effective ecological half-lives and ecological half-lives of ^{137}Cs in water in the tributaries and outflow of the Orlík Reservoir in the periods 1990–1994 and 1995–2010.

Period	1990–1994		1995–2010	
Tributaries of Orlík Reservoir	***T**$_{eff}$* (y)	***T**$_{ecol}$* (y)	***T**$_{eff}$* (y)	***T**$_{ecol}$* (y)
Vltava River at Hněvkovice	1.5 ± 0.9	1.6 ± 1.0	6.7 ± 1.8	8.5 ± 2.9
Lužnice River at Koloděje	2.2 ± 1.6	2.4 ± 2.0	10.9 ± 4.9	17.0 ± 12.0
Otava River at Topělec	1.5 ± 1.1	1.5 ± 1.3	6.3 ± 2.1	8.0 ± 3.3
The outflow from Orlík Reservoir (the Vltava River at Solenice)	1.5 ± 0.6	1.5 ± 0.7	6.2 ± 1.7	7.8 ± 2.7

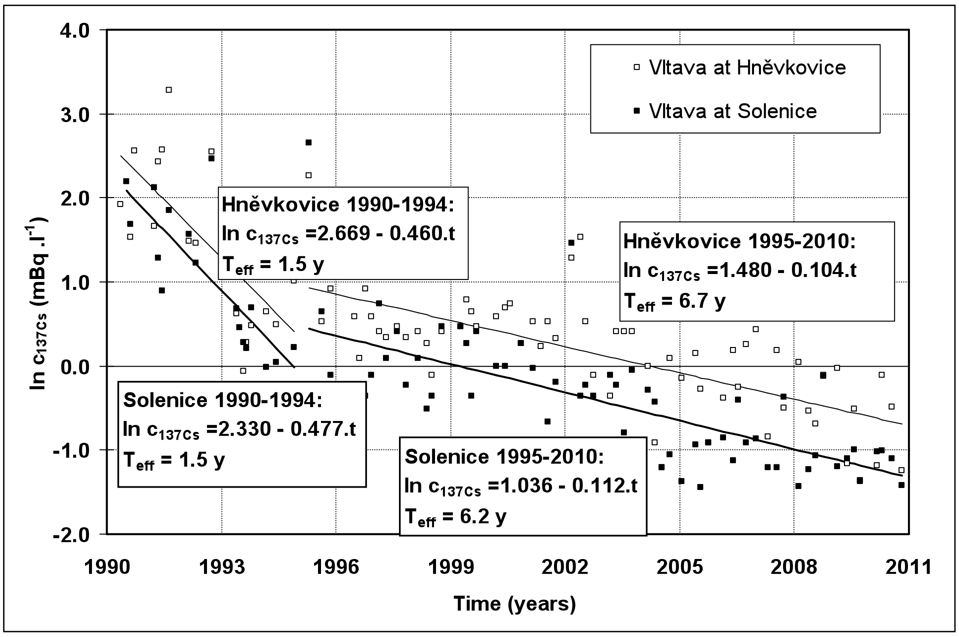

Fig. 2 Temporal changes of ^{137}Cs concentration (c_{137Cs}) in the Vltava River at Hněvkovice (source of technological water) and the Vltava River at Solenice (downstream from the Temelín waste water outflow) in the periods 1990–1994 and 1995–2010.

in 1989–2000. Similarly, the rate of decline in the concentration of ^{137}Cs in the Pripyat River has decreased in recent years (Smith and Beresford 2005). The effective ecological half-lives of 1.2 years (dissolved phase) and 1.7 y (particulate phase) in the period 1987–1991 increased to 3.4 y (dissolved phase) and 11.2 y (particulate phase) in the period 1995–1998. This increase in T_{eff} was also recorded in Belarus, Ukraine and Finland (Smith and Beresford 2005).

Temporal changes in the concentrations of ^{90}Sr in water samples collected from Orlík Reservoir and its tributaries were recorded for the period 1993–2010. The effective ecological half-lives (T_{eff}) in the water from individual tributaries and outflow from Orlík Reservoir (Table 2) were in the range 7.9–12.8 y and the ecological half-lives (T_{ecol}) in the range 10.8–22.9 y. An example is shown in Fig. 3 for the Vltava River at Hněvkovice and Solenice. In 2010, the average activity of ^{90}Sr at Hněvkovice was 2.9 mBq l^{-1} and at Solenice 2.3 mBq l^{-1}. The anthropogenic radionuclides ^{137}Cs and ^{90}Sr in the hydrosphere downstream from where waste water is discharged from the Temelín power plant originated mainly

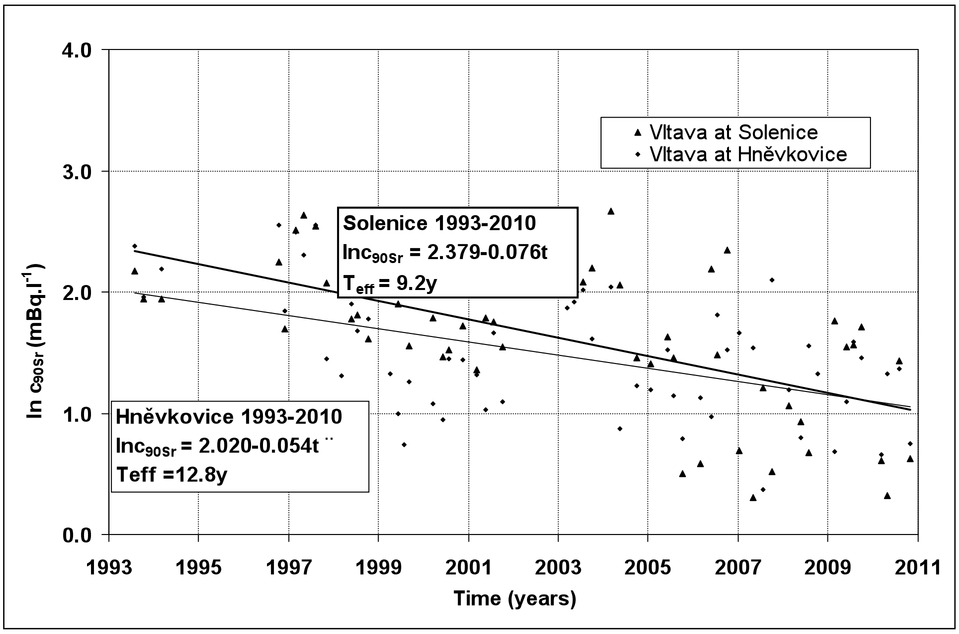

Fig. 3 Temporal changes of ^{90}Sr concentration (c_{90Sr}) in the Vltava River at Hněvkovice and the Vltava River at Solenice in the period 1993–2010.

Table 2 The evaluated effective ecological half-lives and ecological half-lives of ^{90}Sr in water in the tributaries and outflow of the Orlík Reservoir in the period 1993–2010.

Period	1995–2010	
Tributaries of Orlík Reservoir	T_{eff} (y)	T_{ecol} (y)
Vltava River at Hněvkovice	12.8 ± 5.9	22.9 ± 10.6
Lužnice River at Koloděje	7.9 ± 2.7	10.8 ± 3.7
Otava River at Topělec	9.4 ± 3.3	14.0 ± 4.9
The outflow from Orlík Reservoir (the Vltava River at Solenice)	9.2 ± 3.5	13.5 ±5.1

from the residual contamination from tests of nuclear weapons in the atmosphere and the Chernobyl accident. The activity of these radionuclides is decreasing and currently the concentrations in surface water are near their detection limits.

Over the period of this study the concentrations of ^{137}Cs and ^{90}Sr in the tributaries of Orlík Reservoir were similar to that recorded in other European countries that were affected by radioactive fallout from the Chernobyl accident. For example, in Finland and the concentrations of ^{137}Cs recorded in Lago Maggiore and its tributaries in Italy (Putarskaya et al. 2009). The difference in the decline in the concentration of ^{137}Cs and ^{90}Sr is reported for a number of European rivers. Concentrations of ^{137}Cs in the global fallout from the Chernobyl accident after the tests of nuclear weapons in the atmosphere greatly exceeded those of ^{90}Sr. The activities of both radionuclides rapidly declined in the initial period and this trend continued for ^{137}Cs but the rate of decline in the activity of ^{90}Sr has decreased. This is reflected in lengthening of its effective ecological half-life (Smith et al. 2000a). However, the activities of these radionuclides continued to decrease at all the river sites we studied after the Temelín power plant became operational (since 2001).

Concentrations of radiocaesium and radiostrontium in sediments

The concentrations of ^{137}Cs, ^{134}Cs and ^{90}Sr in the sediments were recorded. Over the whole period, the mean concentration of ^{137}Cs in sediments was 73.6 Bq kg^{-1} and in 2001–2010, it was 33.6 Bq kg^{-1}. For the whole of the Czech Republic, the mean ^{137}Cs concentration in the period 2001–2010 was 12.9 Bq kg^{-1} (TGM WRI 2011), which indicates that the sediments in Orlík Reservoir and its tributaries were highly contaminated with ^{137}Cs compared with the rest of the Czech Republic. Mean concentration of ^{90}Sr in the period (1993–2010) of 1.8 Bq kg^{-1} was substantially below that of ^{137}Cs.

The activities of these radionuclides are decreasing in time (Fig. 4). The rates of decline are similar for reference sampling sites and affected river sites and therefore the trends in the decline were evaluated for average annual

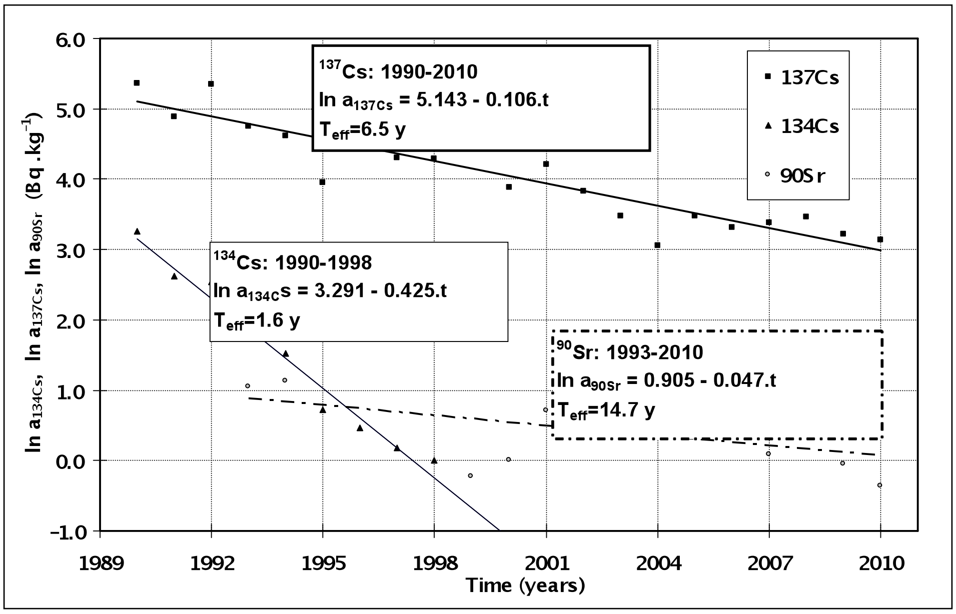

Fig. 4 Temporal changes of annual average concentrations of ^{134}Cs (a_{134Cs}), ^{137}Cs (a_{137Cs}) and ^{90}Sr (a_{90Sr}) in bottom sediments (dry matter) in Orlík Reservoir and its main tributaries in the periods 1990–1998 (^{134}Cs), 1990–2010 (^{137}Cs) and 1993–2010 (^{90}Sr).

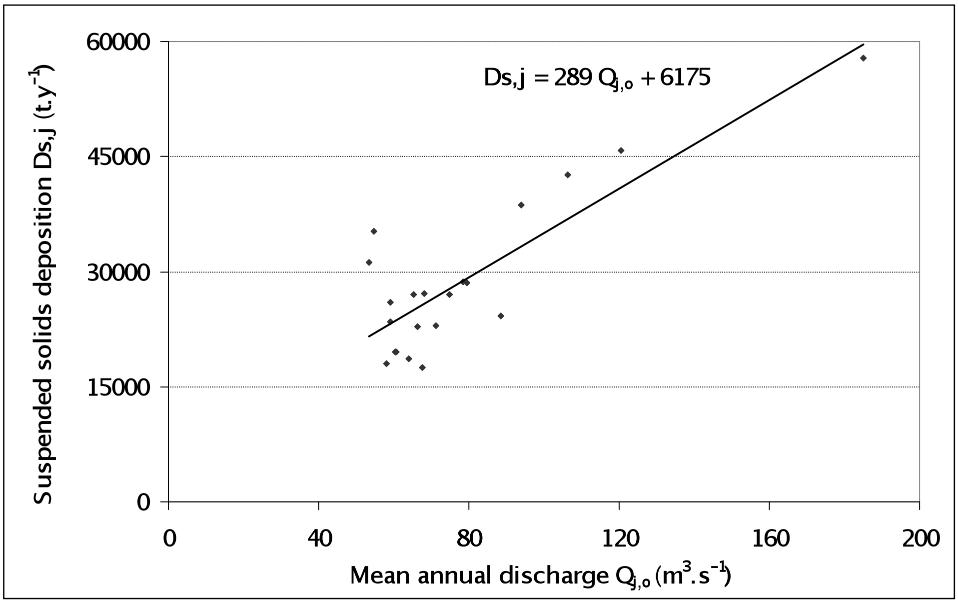

Fig. 5 Dependence of the suspended solids deposition in Orlík Reservoir on the annual mean flow.

activities for all sites. The assessment of ^{134}Cs was stopped in 1998 because in that year the values were below the MDA. The effective ecological half-life was 1.6 y for ^{134}Cs (for the period 1990–1998) and estimated ecological half-life was 7.8 y. For ^{137}Cs, the effective half-life was 6.5 y for the period 1990–2010. The estimated ecological half-life was 8.4 y. For ^{90}Sr, the effective ecological half-life was 26.2 y for the period 1993–2010.

The results of the analysis of sediments showed that the residual contamination from the tests of nuclear weapons in the atmosphere and the Chernobyl accident in the last century is greater than that originating from the waste water from the Temelín power plant. Apart from ^{134}Cs, ^{90}Sr and ^{137}Cs, the results of the monitoring did not indicate that the sediments were contaminated with any other activation and fission products detectable using gamma-spectrometric analysis or ^{90}Sr determination.

Depositions in Orlík Reservoir

Data on river flows and concentrations of suspended solids, ^{90}Sr and ^{137}Cs were used to assess possible effects of the reservoir on the substances monitored.

Annual mean concentrations of suspended solids in samples from Orlík Reservoir and its tributaries were used together with annual mean flows for determining

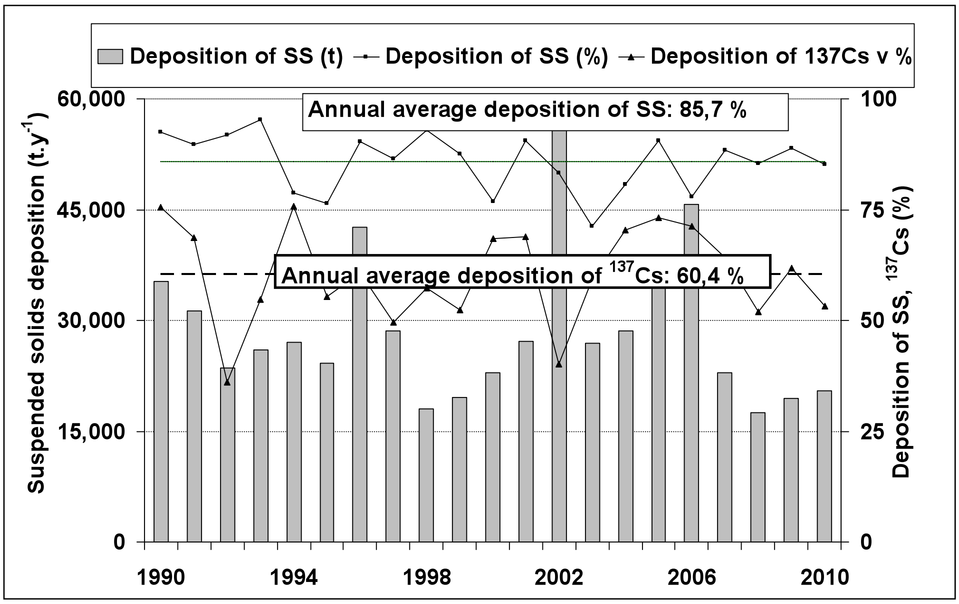

Fig. 6 Annual deposition of suspended solids (SS) and ^{137}Cs (deposited SS expressed in tons and percentages and deposited ^{137}Cs expressed in percentages in the period 1990–2010).

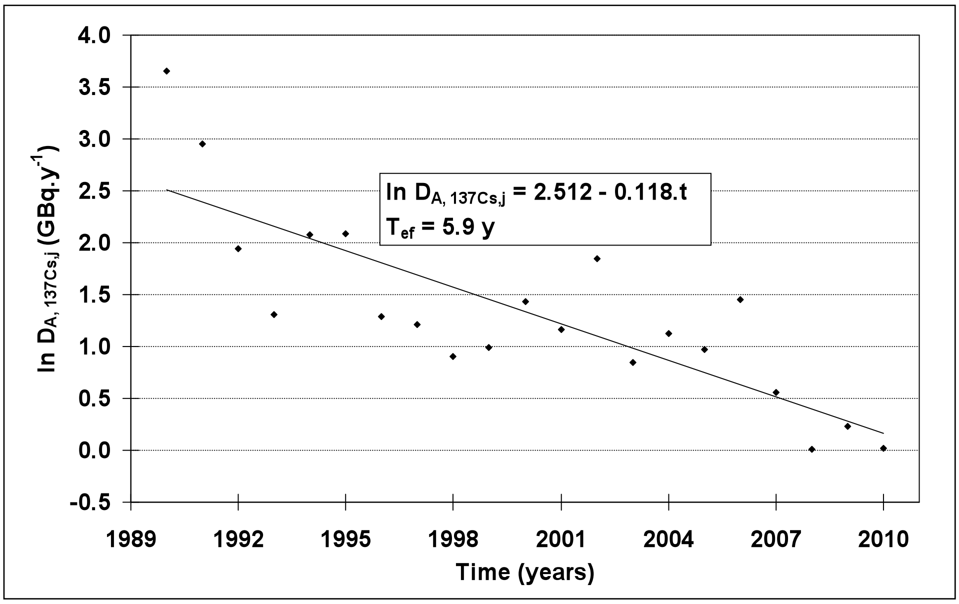

Fig. 7 Decrease in ^{137}Cs deposition in Orlík Reservoir during 1990–2010.

the relationship between the deposition of suspended solids in Orlík Reservoir and annual mean flow (Fig. 5). Subsequently, this revealed that the annual deposition of suspended solids ranged between 71–95% (with an average value of 85.7%) of the inflow of suspended solids. In terms of mass, the annual mean deposition is 28,800 tons. The deposition of suspended matter in Orlík Reservoir expressed in percentages did not show any time dependence.

The annual deposition of ^{137}Cs was between 36% and 76% (1.0–19.2 GBq r^{-1}) and averaged 60.4%. The annual deposition of suspended solids (SS) and ^{137}Cs is presented in Fig. 6. The annual deposition of ^{137}Cs decreased over time (Fig. 7) to a half-life of 5.9 years in the period 1990–2010. The temporal trend in the decrease is similar to that recorded in ^{137}Cs activity in water and bottom sediments in the study area. The mean percentage ^{137}Cs deposition was lower than that of suspended solids. This indicates that a part of the ^{137}Cs is dissolved in water and the deposited component is associated with solid particles. This assumption accords with the high distribution coefficients for ^{137}Cs (Kd) reported for Lake Constance and the river Rhine, which are in the range $4.6 \cdot 10^4$–$2.7 \cdot 10^6$ l kg^{-1} (Smith and Beresford 2005). The decrease in the deposition of ^{137}Cs in the Orlík Reservoir with an effective ecological half-life of 5.9 years accords with its half-life of 6.5 years based on the decrease in annual mean activity of ^{137}Cs in bottom sediments sampled from the reservoir and its tributaries during the period 1990–2010.

The analysis of ^{90}Sr concentrations showed that the outflow from the reservoir exceeds that of the inflow from the tributaries and the inter-basin area. The percentage outflow of ^{90}Sr was in the range −37.8% to 72.1%, with an average value of 19.6%. The outflow of ^{90}Sr from the reservoir corresponds with the higher mobility and lower values of its Kd (750–1800 l kg^{-1}) recorded for the area surrounding the Chernobyl Nuclear Power Plant (Smith and Beresford 2005).

Similar accumulations of ^{137}Cs are reported for the cascade of reservoirs constructed on the Dnepr River (Internet) and Lago di Lugano and Lago di Maggiore in Switzerland and Italy. The concentrations of ^{137}Cs in the upper lake (Lago di Lugano) exceed those in the lower lake (Lago di Maggiore) by one or two orders of magnitude (Putyrskaya et al. 2009). Accumulation of ^{90}Sr in reservoirs was not substantiated (IAEA 2005). During some periods, the ^{90}Sr concentrations in reservoirs can even remobilize and discharge from a reservoir, which is supported by the results for Orlík Reservoir, for which the mean ratio between the inflow and outflow of ^{90}Sr was 0.90.

Outflows of ^{137}Cs and ^{90}Sr were assessed in relation to their concentrations in individual basins after the Chernobyl accident and tests of nuclear weapons in the atmosphere. During the period 1990–2010, the annual outflow of ^{137}Cs from individual basins did not exceed 0.01% of the total activity accumulated in the basin. At the end of this period, the annual outflows in the tributaries of Orlík Reservoir and of the outflow from the reservoir were in the range 0.002% to 0.005%. Similarly, Erlinger et al. (2009) report that in 2005 the outflows from small basins in the Austrian Alps ranged between 0.0008% and 0.0031% with an average of 0.002%. For the whole basin of the Vltava, Lužnice and Otava Rivers upstream from the Vltava River at Solenice, the total outflow of ^{137}Cs in the period 1986–2010 was 0.5% of the ^{137}Cs activity accumulated in the basin, which confirms that the outflow of ^{137}Cs contributes little to the total reduction in ^{137}Cs in this basin.

The contribution of the outflow of ^{90}Sr to its total reduction in the basin exceeds that of ^{137}Cs by approximately one order of magnitude. During the period 1986–2010, the outflow of ^{90}Sr represented 3.3% of that present in the basin of the Vltava River at Solenice. This is similar to the results reported by Saxén and Ilus (2001), who conclude that the contribution of the outflow of ^{90}Sr exceeds that for ^{137}Cs because immobilization of ^{90}Sr in sediments is much less than the fixation of ^{137}Cs.

Bioaccumulation

The monitoring of aquatic plants and fish focused on determining the concentrations of ^{137}Cs and ^{90}Sr. The concentrations of ^{137}Cs in fish (in terms of fresh weight) were assessed for the periods 1986–1990 and 1994–2010. Between the two periods, the ^{137}Cs concentrations decreased from 2.0–51.4 Bq kg^{-1} (1986–1990) to 0.08–2.00 Bq kg^{-1} (1994–2010). As there was less data available on the concentrations of ^{90}Sr in fish the value was assessed for the whole period 1990–2010, for which the mean concentration was 0.8 Bq kg^{-1}. The results of the monitoring and assessment of the ^{137}Cs and ^{90}Sr concentrations in fish are presented in Fig. 8. The concentrations in the Czech Republic are substantially below those in areas affected by the first radioactive cloud. In the most affected areas in the vicinity of Chernobyl, these activities shortly after the accident were at levels of hundreds of kBq kg^{-1} and in the early nineties were still at levels of tens of kBq kg^{-1}. Activities of several Bq kg^{-1} in this period were also reported from Switzerland, England and Germany (Smith et al. 2000b).

Relatively rare information on ^{90}Sr concentrations in fish include those of Outola et al. (2009), who report that in the period 1978–1997 the concentrations in the river species they analyzed were in the range of 10–17 Bq kg^{-1}, which exceeds the ^{90}Sr concentrations in fish from Orlík Reservoir by approximately one order of magnitude. The concentration levels of ^{90}Sr were however smaller by several orders of magnitude compared to those for ^{137}Cs. Most of the ^{90}Sr is in bones and thus it is less dangerous than ^{137}Cs in terms of radioactive doses originating from the food chain (Outola et al. 2009).

In accord with the results for surface water, the concentrations of ^{137}Cs and ^{90}Sr in fish also decreased over time. Evaluated effective ecological half-life (T_{eff}) of ^{137}Cs was 1.0 y for the period 1986–1990 and 4.5 y for the period 1994–2010 and that of the ecological half-lives (T_{ecol}) 1.1 y and 5.4 y, respectively. Similar results are reported by Franić and Marović (2007) for Croatia over the period 1987–1992, while this decrease is greater than that reported by Smith et al. (2000b) for the same period. The reported half-lives are in the range 2 to 3 years. In accord with the results for the Czech Republic, the literature indicates that the decrease in the following period was significantly less when expressed in terms of physical half-life. The effective ecological half-life in the Finland lakes is between 3 and 6 years (Outola et al. 2009). Franić and Marović (2007) report 5 years for the period 1993–2005. The ^{137}Cs half-life for fish is similar to that for water. The decrease continued also during the operation of the Temelín power plant.

The half-life reported for ^{90}Sr is substantially longer. Evaluated effective ecological half-life (T_{eff}) for ^{90}Sr was 6.4 y for the period 1990–2010 and ecological half-life (T_{ecol}) 8.2 y. The effective ecological half-lives for several species of fish in Finish lakes range between 7 and 30 years (Outola et al. 2009).

The results of monitoring the ^{137}Cs and ^{90}Sr in surface water and fish (fresh weight) were used to determine

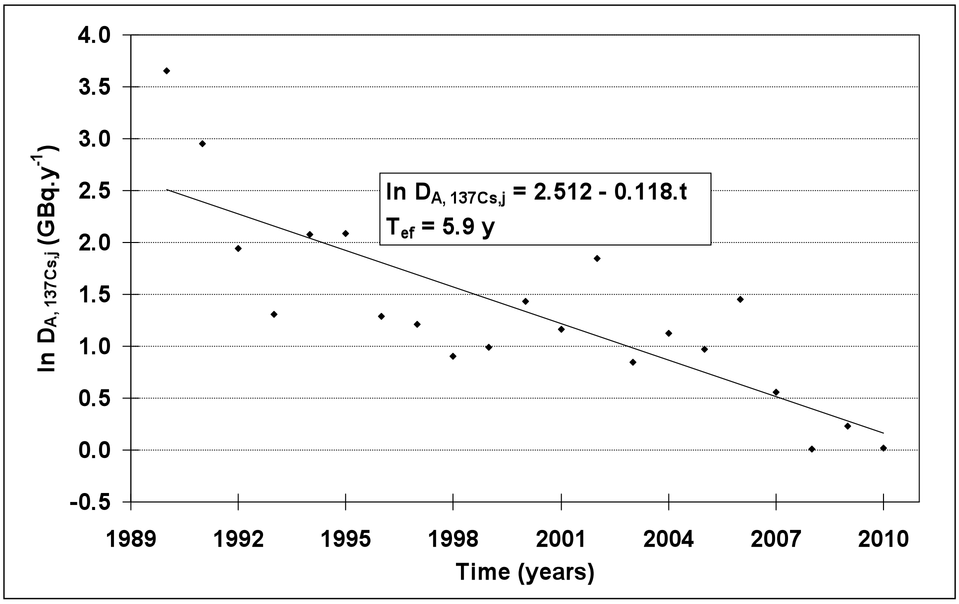

Fig. 8 Temporal changes in ^{137}Cs and ^{90}Sr concentrations (*a*137Cs, *a*90Sr) in fish (wet weight) in Orlík Reservoir in the periods 1986–1990, 1994–2010 (*a*137Cs) and 1990–2010 (*a*90Sr).

the concentration factors CF_{137Cs} and CF_{90Sr}. Outola et al. (2009) conclude that the concentration factor can be calculated when conditions have stabilized, which occurs 8 to 10 years after an accident. For Orlík Reservoir, the CF_{137Cs} values calculated for the period 1986–1990 did not differ significantly from those for the period after 1994 and therefore the mean CF_{137Cs} was calculated using all the values. The resulting value was 309 l kg^{-1}. The mean CF_{137Cs} value specified in IAEA (2010) is $3.0 \cdot 10^3$ l kg^{-1} and the range $4.5 \cdot 10^1$–$2.4 \cdot 10^4$ l kg^{-1}, whereas the mean ^{137}Cs for fish species in Orlík Reservoir is smaller by one order of magnitude. All of the values calculated were, however, in the range reported by IAEA (2010).

For ^{90}Sr, the mean concentration factor (CF_{90Sr}) was 124 l kg^{-1}, which is similar to the mean of $1.9 \cdot 10^2$ l kg^{-1} (range of $2.2 \cdot 10^1$–$7.1 \cdot 10^2$ l kg^{-1}) reported in IAEA (2010). Similar results are reported by Smith and Beresford (2005) for lakes in the vicinity of Chernobyl (46–452) while Outola et al. (2009) report 550–1300 l kg^{-1} for lakes in Finland.

Concentrations of ^{137}Cs were monitored in several species of aquatic plants (dried material). The results substantiated the assumption that the highest concentrations of ^{137}Cs occur in aquatic mosses (21.8 Bq kg^{-1} in 1996) and algae (17.9 Bq kg^{-1} in 1996). A comparison of the results for sites unaffected and affected by the outflow from the Temelín power plant was complicated because different species of plants grew at the individual sites with exception of reeds. Since 2006, the monitoring was confined to reeds, which were also used for the assessment. The results indicate that the concentrations of ^{137}Cs in reeds decreased with the effective ecological half-life 13.5 years. The decrease occurred at both the unaffected and affected river sites and continued after the Temelín power plant became operational. The ^{90}Sr concentration in reeds ranged between 0.5 and 6.1 Bq kg^{-1} (in dry matter) and this concentration decreased with an effective ecological half-life of 6.9 years.

A summary of the effective ecological half-lives and ecological half-lives of ^{134}Cs, ^{137}Cs, and ^{90}Sr and the concentration factors for individual components of the hydrosphere is presented in Table 3. The periods of time over which the assessments were made are given in detail in the above text.

Conclusions

The results of the systematic monitoring of possible effects of the Temelín power plant on the hydrosphere show that the waste water discharges meet the limits specified in the permit on water management (Decision of Regional Authority – Permit on Water management 2007) and the Resolution of the Czech Government No. 229/2007 Coll. Concentrations of anthropogenic radionuclides in the hydrosphere downstream from the waste water outflow from the Temelín power plant are mainly due to the residual contamination from global fallout and the Chernobyl accident. Apart from tritium, the effects of the Temelín power plant on the concentration of activation and fission products in the hydrosphere were negligible.

For two periods of time, 1990–1994 and 1995–2010, the concentrations of ^{137}Cs in surface water and fish, and for one period, 1990–2010, in sediments and in 1996–2010, in aquatic plants. Its effective ecological half-life in water in individual tributaries and the outflow from Orlík Reservoir were in the range 1.1–2.2 years for 1990–1994 and 6.2–10.9 years for 1995–2010. These results indicate that in the first period, which is shortly after the accident at Chernobyl, the concentrations of ^{137}Cs were rapidly decreasing and continued to decline but at a slower rate in the second period (after 1995). Its effective ecological half-life in fish was 1.0 y for the period 1986–1990 and 4.5 years for the period 1994–2010. Concentrations of ^{137}Cs in water and fish decreased at approximately the same rate. Temporal changes in the concentrations of ^{134}Cs and ^{137}Cs in sediments were recorded for the periods 1990–1998 and 1990–2010. The monitoring of ^{134}Cs stopped in 1998 because in that year all the

Table 3 Summary of the effective ecological and ecological half-lives of ^{134}Cs, ^{137}Cs and ^{90}Sr, and concentrations in different components of the hydrosphere (The time periods over which these were assessed are given in text).

	^{134}Cs				^{137}Cs					^{90}Sr				
	T_{eff} (y)		T_{ecol} (y)		T_{eff} (y)		T_{ecol} (y)		CF 1/kg	T_{eff} (y)		T_{ecol} (y)		CF 1/kg
Water	–				1.7	± 2.1	1.8	± 2.4	–	9.8	± 7.6	15.0	± 12.0	–
					7.5	± 5.1	10.3	± 9.8						
Sediment	1.6	± 0.3	7.8	± 6.0	6.5	± 1.4	8.4	± 2.3	–	15.0	± 21.0	30.0	± 87.0	
Fish	–				1.0	± 0.2	1.1	± 0.2	310	6.4	± 4.0	8.2	± 6.6	124
					4.5	± 1.5	5.3	± 2.1	± 190					± 80
Flora	–				14	± 13	25	± 43.0	64	6.9	± 2.8	9.1	± 4.9	20
(Reeds)									± 30					± 9

values were below the MDA. The effective half-life was 1.6 y for ^{134}Cs in the period 1990–1998 and 6.5 y for ^{137}Cs in 1990–2010. The values for the concentration of ^{137}Cs in reeds revealed that it was decreasing with an effective ecological half-life of 13.5 years. This decrease was recorded in both unaffected and affected rivers and continued even after the Temelín power plant became operational.

Temporal changes in the concentrations of ^{90}Sr in water collected from Orlík Reservoir and its tributaries were recorded for the period 1993–2010. The effective ecological half-life in water in individual tributaries and the outflow from Orlík Reservoir were in the range 7.9–12.8 y. Temporal changes in the concentrations of ^{90}Sr in sediments were recorded for the period 1993–2010. The effective ecological half-life was 26.2 y. The concentration of ^{90}Sr in reeds decreased over this period and its effective ecological half-life was 6.9 years.

The differences in the effective half-lives recorded at the different sites are due to differences in the conditions at these sites. They can differ from each other, for example, in flow velocity, concentration of suspended solids or quality of the sediments.

Data on river flow and concentrations of suspended solids were used to assess possible effects of the reservoir on the monitoring of ^{90}Sr and ^{137}Cs. The annual deposition of suspended solids ranged between 71–95% (with an average value of 85.7%) of that in the inflow. The annual deposition of ^{137}Cs was between 36% and 76% with an average value of 60.4%. The annual deposition of ^{137}Cs decreased over time and its half-life in the period 1990–2010 was 5.9 years. The analysis of the concentrations of ^{90}Sr revealed that there was more in the outflow from the reservoir than in the inflow from the tributaries and inter-basin area. The percentage outflow of ^{90}Sr was in the range −37% to 72% with an average of 20%.

During the period 1990–2010, the concentration factor for ^{137}Cs in fish was on average 309 l k^{-1}g.

The concentrations of ^{137}Cs and ^{90}Sr in the surface water, river bottom sediments, fish and aquatic flora collected from the vicinity of the Temelín power plant decreased over the period of this study, even in the samples collected downstream from the waste water outflow from the Temelín power plant.

Acknowledgements

This paper was prepared from the results of project MZP 0002071101, which was sponsored by the Ministry of the Environment of the Czech Republic.

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FEASIBILITY OF VERMICOMPOSTING DEWATERED SLUDGE FROM PAPER MILLS USING *PERIONYX EXCAVATUS*

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ABSTRACT

India has a large network of pulp and paper mills of varying capacity. On an industrial scale the sludge from paper and pulp mills is disposed of either as landfill or incinerated. Both methods result in the loss of a valuable resource and have obvious environmental and economic disadvantages. The solid waste from pulp and paper mills is a source of organic matter and its proper disposal and management is the responsibility of the industry. As composting/vermicomposting could be used to transform this waste trials were carried out to determine the feasibility of converting dewatered sludge (DS) into a value added end product using an earthworm, *Perionyx excavatus*. The vermicomposting of the waste resulted in an increase in its electrical conductivity (EC), ash content, total nitrogen (TN), total phosphorous (TP) and available phosphorous (AP), respectively, and a decrease in total organic carbon (TOC), biochemical oxygen demand (BOD), chemical oxygen demand (COD), oxygen uptake rate (OUR) and evolution of carbon dioxide (CO_2). Overall, the best treatment was T5 in which there was a 76.1% increase in TP, 58.7% in TN, 74.5% decrease in TOC, and a reduction of 6.7 fold in the production of CO_2 and 10.7 fold in BOD, respectively. Our trials demonstrate that vermicomposting using an epigeic earthworm, *Perionyx excavatus*, is an alternate and environmentally safe way of recycling paper mill sludge if it is mixed with an appropriate amount of cow dung and food processing waste. Overall T5 was the best combination of paper mill sludge and waste for vermicomposting followed by T3, T2, T4 and T1, respectively.

Keywords: C/N, vermicompost, food processing waste, cow dung

Introduction

Disposal of industrial sludge using environmentally acceptable means poses a very great challenge worldwide. It has been suggested that earthworms be used for this purpose (Elvira et al. 1998) and the process has been termed vermistabilization (Neuhauser et al. 1988). A considerable amount of work has been done on the composting of various organic materials using earthworms, which has revealed that epigeic earthworms can hasten the composting process to a significant extent and produce a better quality of compost than that produced by traditional composting methods (Ghosh et al. 1999). *Perionyx excavatus* is an epigeic species of earthworm, which lives in organic waste and could be potentially used to convert organic waste into a valuable end product. *P. excavatus* is known to be good at converting organic waste into high-value vermicompost, which can be used as a medium for growing plants (Kale et al. 1982; Suthar 2006). Nevertheless, most of the vermiculture experiments using *P. excavatus* were done using animal dung e.g. cow dung (Kale et al. 1982; Reinecke et al. 1992; Edwards et al. 1998), sheep dung (Kale et al. 1982), biogas sludge (Kale et al. 1982; Edwards et al. 1998), poultry manure (Kale et al. 1982), pig solids (Edwards et al. 1998), horse solids (Edwards et al. 1998) and turkey waste (Edwards et al. 1998). The potential of *P. excavatus* for processing other wastes, namely vegetable waste (Singh et al. 2005) and water hyacinth (*Eichhornia crassipes*) (Gajalakshmi et al. 2001), however, has also been tested. Vermicomposting is not only rapid, easily controllable, cost effective, energy saving and a zero discharge process, but also efficiently accomplishes the recycling of organic substances and nutrients. Transformation of organic industrial waste by vermicomposting can be the cheapest and safest way of disposing of it without polluting the environment (Elvira et al. 1996; Kaushik and Garg 2003) and recovering vermi fertilizer and animal protein (Chaudhuri 2005). Moreover, vermicompost is fragmented and microbially active due to humification (Edwards and Bohlen 1996; Maboeta and Rensburg 2003) and contains important plant nutrients in forms that are soluble and more easily available to plants than those in ordinary compost (Ndegwa and Thompson 2001). Therefore, the objective of this study was to test the feasibility of using the earthworm *P. excavatus* to stabilize dewatered sludge (DS) from pulp and paper mills mixed with cow dung (CD) and food processing waste (FPW) in different ratios.

Materials and methods

Earthworm cultures

The species of earthworm chosen for the compositing experiment was *Perionyx excavatus*. This earthworm was obtained from Central Plantation Crops Research Institute (CPCRI), Indian Council of Agricultural Research, Guwahati, India. For rearing the earthworm cultures, hopper bottom Perspex bins, 450 mm × 300 mm × 450 mm in size, were fabricated in the laboratory. For aeration and drainage purposes 16 holes of 10 mm diameter were drilled along the longer sides and 16 in the bottom, respectively. Hoppers were used to collect leachate (if any). Before the addition of the culture medium and earthworms, bedding was prepared from partially degraded chopped hay (about 50 mm), cow dung, banana pulp (chopped about 50 mm) and tree leaves. This bedding was watered to keep it moist to enable the worms to breathe. Then the earthworms were added along with partially degraded cow dung as a source of food for the earthworms.

Compost material

Dewatered sludge (DS), cow dung (CD) and food processing waste (FPW) were mixed in different proportions. DS was collected from the *effluent treatment plant* (ETP) of Nagaon Paper Mill, Kagajnagar, Assam (India). The dewatered sludge, as the name suggests, was collected from the ETP plant after the waste had passed through the sedimentation process and then partially dewatered by pumping. CD was obtained from a livestock farm near IIT, Guwahati campus, Assam (India) and FPW from Bhogali Jalpan (a traditional breakfast item maker), Guwahati, Assam (India). The percentages and physico-chemical properties of DS, CD and FPW in the different mixtures are reported in Table 1.

Experiment

Locally made round bamboo containers, each with a radius of 120 mm and depth of 90 mm enclosing a volume of 90.47×10^4 mm^3, were filled with mixtures containing different percentages of DS, CD and FPW. There were three replicas of each treatment (Table 1). The containers were kept in the laboratory at room temperature and the total weight of substrate in each container was kept at 1.5 kg. There was 10 cm of bedding in each container consisting of a mixture of hay (155 g), CD (375 g), banana leaves and tree leaves (280 g), which had been previously partially degraded over a period of two weeks. Approximately 50 g or ~100–120 earthworms (*P. excavatus*), consisting of both mature and juvenile individuals, were placed on the bedding and left to acclimatize to the new environment and then the next day the substrate was placed on top of the bedding.

1.5 kg of five different mixtures of DS, CD and FPW was added to each of the containers and they are referred to as T1, T2, T3, T4 and T5, respectively. In addition there was a control for each mixture CT1, CT2, CT3, CT4 and CT5, respectively. The quantity of the substrate provided was based on the fact that the earthworms can consume half their body weight per day of substrate under favourable conditions (Haimi and Huhta 1987). The moisture level was maintained throughout the study period by periodically sprinkling tap water over the substrate. To pre-

Table 1 The weights and the initial characteristics of the different wastes in the compost.

Reactors/Parameters	Waste materials		
	Dewatered sludge (DS)	Cow dung (CD)	Food processing waste (FPW)
T1 (kg)	1.5	–	–
T2 (kg)	1.0	0.24	0.26
T3 (kg)	1.0	0.34	0.16
T4 (kg)	1.0	0.41	0.09
T5 (kg)	1.0	0.46	0.04
Moisture content (MC) (%)	71.2±1.2	83.1±3.2	9.2±2.6
pH	6.82±0.01	7.20±0.03	6.61±0.02
Electrical conductivity (mmohs/cm)	0.73±0.02	1.21±0.02	1.22±0.04
Ash content (%)	43.1±1.4	27.5±1.2	6.6±0.4
Total organic carbon (TOC) (%)	31.6±1.7	40.3±2.1	51.8±1.5
Total nitrogen (TN) (g/kg)	6.4±0.8	15.1±1.1	16.4±0.9
Ammonical Nitrogen (NH4-N) (mg/kg)	3.21±1.02	4.62±1.21	3.12±1.34
Total phosphorous (TP) (g/kg)	3.91±0.41	0.62±0.12	1.21±0.32
Available phosphorus (AP) (g/kg)	0.37±0.11	0.22±0.09	0.01±0.01
Chemical oxygen demand (COD) (mg/kg)	591±27	145±16	22736±341
Biochemical oxygen demand (BOD) (mg/kg)	370±41	110±17	16±8
CO2 evolution (mg/g VS/day)	8.72±0.84	22.41±1.20	10.74±0.59
Oxygen uptake rate (OUR) (mg/g VS/day)	18.9±0.5	40.1±1.1	1.0±0.1

vent moisture loss, the bamboo containers were covered with gunny bags.

Parameters measured

About 110 g of wet substrate (free of earthworms, hatchlings and cocoons) were collected from the containers on day zero and on the 15th, 30th and 45th day of the composting period. Day zero is the day before earthworm inoculation. 10 g of the sample was used to measure soluble biochemical oxygen demand (BOD), chemical oxygen demand (COD), oxygen uptake rate (OUR) and CO_2 production as described in Khwairakpam and Bhargava (2009a). Sub-samples were oven dried, ground to pass through a 0.2-mm sieve and stored in plastic vials for further analysis: pH and conductivity were measured in 1 : 10 (w/v) water suspensions using digital pH (μ pH system 361) and conductivity meters (VSI-04 Deluxe), ash content (550 °C for 2 h) (loss on ignition), total nitrogen (TN) using the Kjeldahl method, ammonical nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) using KCL extraction (Tiquia and Tam 2000), total organic carbon (TOC) determined by Shimadzu (TOC-V_{CSN}) Solid Sample Module (SSM-5000A) and total and available phosphorus (TP and AP) using acid digestion and the stannous chloride method (APHA 2001). The C/N value was calculated from the measured values of total organic carbon and nitrogen. In addition earthworm growth in terms of biomass and total mortality were measured at the end of every 15th day of the experiment.

Statistical analysis

All the results are the means of three replicates. The results were statistically analyzed at 0.05 probability level using one way analysis of variance (ANOVA) and Tukey's HSD test as a post-hoc analysis to compare the means using Statistica software.

Results and discussion

Moisture content

In most treatments the moisture content increased during decomposition. The exceptions were T1, CT1 and T5. It is important that the moisture content is suitable for the earthworms and micro-organisms in the vermicomposting system and in this experiment it was 64–67% in most of the treatments. Lower moisture contents were recorded in T1 (8%) and CT1 (4%), which might be due to the nature of the raw waste material (100% DS). The greatest changes in water content were recorded in T2 (15.7%) and CT2 (17%) at the end of 45 days (Table 1). The ideal moisture range for vermicomposting or vermiculture is 60–80% (Neuhauser et al. 1988; Edwards 1998), which was achieved in this experiment. The variations in moisture content during the vermicomposting were significant ($P < 0.05$).

pH

The value of pH increased in all the treatments. The greatest increase in pH was recorded in T3, where it increased from an initial value of 5.7 to 7.7, followed by CT3 (5.7 to 7.1) and the lowest increase was recorded in CT1 (6.8 to 6.9) followed by CT2 (6.7 to 7.2) (Table 1). That is, the change in pH in all treatments indicated the compost became more alkaline and suitable for application to soil. Compost has a liming effect due to its richness in alkaline cations such as Ca, Mg and K, which were liberated from OM due to mineralization. Consequently, regular applications of compost maintain or enhance soil pH (Ouedraogo et al. 2001). Only in a few cases is a decrease in pH recorded after applying compost (Zinati et al. 2001). The change in pH during vermicomposting depends upon substrate, as different substrates may produce different intermediate organic acids (Gupta and Garg 2008). Similar observations are also recorded for vermicomposting by earlier workers (Hait and Tare 2010; Kaur et al. 2010). Earthworms selectively increase populations of catabolically more active microbes (Aira et al. 2007) therefore the degradation of short chain fatty acids and precipitation of calcium carbonate may be the cause of the increase in pH recorded in vermicomposting (Tognetti et al. 2005). Variations in pH were statistically significant ($P < 0.05$).

Electrical conductivity (EC)

The release of different mineral salts in available forms may account for the increase in EC recorded in all the treatments. Greatest percentage change was recorded in T1 (59.2%) and T2 (45.8%) followed by CT1 (32.4%) and the least in CT5 (4.5%), CT4 (6.2%) and CT3 (7.1%), respectively (Table 1). A similar increase in EC is also reported by other authors (Suthar 2007; Gupta and Garg 2008; Yadav and Garg 2009). In a closed system there is an increase in mineral salts associated with the loss in terms of weight of organic matter, which may account for the increase in EC (Khwairakpam and Bhargava 2009a). Variations in EC were statistically significant ($P < 0.05$).

Ash content (%)

The high increase in ash content indicates that the organic material is being degraded during by the vermicomposting process. The highest increase in ash content was recorded after 30 days of vermicomposting. This shows that earthworms consumed the waste material and microbes were active during the decomposition process. The ash content is an important indicator of decomposition and mineralization of the substrate (Gupta et al. 2007; Gupta and Garg 2008; Khwairakpam and Bhargava

Table 2 Moisture content, pH and EC recorded at 15 day intervals during vermicomposting.

Reactors	Moisture content (%)				pH				EC (mmhos/cm)			
	0 day	15 day	30 day	45 day	0 day	15 day	30 day	45 day	0 day	15 day	30 day	45 day
R1	70.6±1.6ad	68.6±2.4a	65.5±2.8a	65.3±1.4a	6.82±0.02a	6.70±0.30ab	7.07±0.15a	7.47±0.30ab	0.73±0.01a	1.00±0.04a	1.33±0.04a	1.79±0.02a
CR1	70.0±2.2d	68.4±2.4a	68.2±3.1a	67.2±1.2a	6.82±0.02a	7.00±0.25b	7.47±0.06a	6.94±0.02b	0.73±0.01a	0.97±0.05a	0.88±0.03g	1.08±0.01e
R2	56.0±3.1b	65.0±2.1ab	67.0±2.2a	66.5±1.5a	6.71±0.01a	6.55±0.42ab	7.32±0.05a	7.50±0.08ab	1.37±0.01b	2.17±0.02c	2.11±0.02c	2.53±0.04c
CR2	56.0±2.2b	65.2±2.2ab	67.6±1.5a	67.6±2.5a	6.70±0.01a	6.74±0.05ab	7.53±0.03a	7.25±0.30ab	1.37±0.02b	2.08±0.02f	1.89±0.02ed	1.98±0.04f
R3	62.0±2.2bc	60.0±1.5b	63.5±1.6a	64.2±2.1a	5.75 ±0.03a	6.37±0.06a	7.53±0.07a	7.70±0.40ab	1.90±0.01c	1.91±0.02be	1.76±0.02b	2.19±0.06d
CR3	62.6±2.2bc	61.0±1.5bc	64.2±2.4a	65.0±2.0a	5.70±0.01a	6.46±0.08ab	7.00±0.07a	7.14±0.40b	1.83±0.01cd	1.87±0.03b	1.67±0.02h	1.97±0.02f
R4	63.1±2.3bcd	60.0±1.8b	62.3±1.5a	64.5±3.0a	6.50±0.02a	6.57±0.20ab	7.14±0.50a	7.56±0.04ab	1.80±0.01d	1.96±0.04e	1.82±0.02be	2.51±0.03c
CR4	63.5±1.5acd	61.0±2.1bc	66.1±2.5a	66.0±1.5a	6.50±0.02a	6.51±0.05ab	7.56±0.32a	7.09±0.45b	1.81±0.01d	1.87±0.02b	1.90±0.01d	1.93±0.03f
R5	65.1±2.1acd	65.0±2.3ab	65.3±3.6a	65.0±2.0a	6.68±0.02a	6.64±0.06ab	7.37±0.40a	7.99±0.25a	2.26±0.01e	2.31±0.03d	2.40±0.03f	2.65±0.02b
CR5	65.4±4.1acd	66.0±1.7ac	67.0±2.2a	66.6±3.0a	6.56±0.03a	6.76±0.05ab	7.52±0.50a	7.41±0.30ab	2.29±0.01e	2.29±0.02d	2.32±0.02i	2.40±0.01g

Mean values followed by different letters in columns are statistically different (ANOVA; Tukey's test, $P < 0.05$)

Table 3 Ash content, TOC and TN content recorded at 15 day intervals during vermicomposting.

Reactors	Ash content (%)				TOC (%)				TN (g/kg)			
	0 day	15 day	30 day	45 day	0 day	15 day	30 day	45 day	0 day	15 day	30 day	45 day
R1	43.1±0.1a	44.2±0.8a	48.2±1.2ac	54.1±1.0ac	31.6±0.8a	31.0±0.1a	28.8±1.1a	25.5±1.0a	6.44±1.20a	7.00±0.50ac	8.50±1.00a	10.90±0.60ae
CR1	43.1±0.1a	44.0±0.5a	46.3±0.5c	53.0±0.6c	31.6±0.1a	31.1±0.1a	29.9±1.2a	26.1±1.1a	6.44±0.83a	6.80±0.50cd	7.00±1.00a	7.70±0.20d
R2	42.8±0.2a	45.3±1.2a	50.5±1.5a	56.5±1.0a	31.8±0.2a	30.4±1.1a	27.5±0.6a	24.2±0.6ac	10.64±0.65b	11.50±1.00b	13.50±1.20b	17.00±1.10b
CR2	42.6±0.2a	43.6±0.3a	48.1±1.1ac	53.8±1.0ac	31.9±0.2a	31.4±0.1a	28.8±1.0a	25.7±0.2a	10.64±0.80b	10.60±0.08be	11.00±0.60cd	11.60±0.10ae
R3	49.8±0.2b	51.3±0.5b	56.3±1.2b	62.9±1.2bf	27.9±0.2c	27.1±0.1c	24.3±0.6b	20.6±1.0bf	9.52±0.43b	11.20±1.00b	13.30±0.80bc	16.50±0.82b
CR3	49.7±1.2b	51.0±0.2b	55.2±1.1b	60.3±0.6b	27.9±1.2c	27.2±0.1c	24.9±1.1b	22.0±1.0bc	9.52±0.50b	10.80±1.00b	11.00±0.20cd	11.60±0.15ae
R4	56.6±1.1c	59.5±1.0c	62.3±1.0de	68.9±0.5d	24.1±1.1b	22.5±1.0b	21.0±0.3cd	17.3±1.1df	7.14±1.10a	9.50±1.10ab	11.20±1.10bcd	14.20±1.12c
CR4	56.9±1.0c	58.2±1.0c	60.5±1.0e	63.7±1.0f	24.0±1.0b	23.2±0.2b	21.9±1.0bc	20.2±0.5bg	7.14±1.00a	8.20±0.80acde	9.00±0.50ad	9.60±0.20ed
R5	58.7±1.7c	60.2±1.2c	65.2±0.5d	76.3±1.5e	23.0±1.7b	22.1±0.5b	19.3±1.0d	13.2±1.0e	5.32±0.40a	9.40±1.20ab	10.90±0.50d	12.90±1.30ac
CR5	58.8±2.0c	59.6±1.0c	61.5±0.2e	65.6±0.3f	22.9±2.0b	22.5±0.1b	21.4±0.3cd	19.1±0.4fg	5.32±0.33a	6.80±1.00c	7.70±0.50a	8.50±0.40d

Mean values followed by different letters in columns are statistically different (ANOVA; Tukey's test, $P < 0.05$)

Table 4 NH_4-N, NO_3-N and TP recorded at 15 day intervals during vermicomposting.

Reactors	NH_4-N (mg/kg)				NO_3-N (mg/kg)				TP (g/kg)			
	0 day	15 day	30 day	45 day	0 day	15 day	30 day	45 day	0 day	15 day	30 day	45 day
R1	32.00±2.40a	0.68±0.05a	ND	ND	ND	ND	15.28±1.10a	114.20±3.50a	3.92±0.30a	5.20±1.30abcd	6.45±1.00acdf	7.15±0.60ad
CR1	32.00±3.50a	0.72±0.02ac	ND	ND	ND	ND	3.00±0.50b	70.00±1.50b	3.92±0.45a	4.00±0.04bc	4.35±0.20f	4.75±0.30d
R2	5.02±1.80b	0.70±0.01ac	ND	ND	ND	ND	54.58±1.60c	186.20±4.60c	4.29±0.16c	5.45±0.08ac	11.32±1.20bc	16.85±1.00b
CR2	5.02±2.20b	0.79±0.02bc	ND	ND	ND	ND	1.70±0.30b	85.40±1.20g	4.29±0.30c	3.25±0.08b	6.45±0.60afg	10.5±2.00ae
R3	5.18±3.20b	0.67±0.04a	ND	ND	ND	ND	74.40±4.00d	154.00±2.50d	4.12±0.25bf	6.55±0.50a	10.28±1.00be	14.42±1.20bc
CR3	5.18±2.10b	0.75±0.02ac	ND	ND	ND	ND	15.24±1.80a	64.00±1.80b	4.12±0.41f	5.26±1.20abcd	8.12±0.05deg	12.22±1.10cef
R4	4.58±3.20b	0.86±0.01bd	ND	ND	ND	ND	138.23±3.10e	252.00±5.00e	4.02±0.80de	6.82±0.60a	10.22±1.50be	15.00±1.20bc
CR4	4.58±2.20b	0.92±0.06d	ND	ND	ND	ND	14.93±1.00a	71.50±3.00b	4.02±0.32bde	5.24±0.45abcd	8.60±1.10bcg	12.45±1.00cef
R5	4.35±1.20b	0.76±0.02ac	ND	ND	ND	ND	148.12±5.00f	442.20±5.00f	3.85±0.45de	5.44±1.10acd	10.24±1.30be	16.15±1.50b
CR5	4.35±2.00b	0.86±0.04bd	ND	ND	ND	ND	54.36±2.30c	162.49±2.10d	3.85±0.18d	4.23±0.50bcd	8.25±0.40aeg	14.50±1.00bf

Mean values followed by different letters in columns are statistically different (ANOVA; Tukey's test, $P < 0.05$) ND-Not detected

Table 5 AP, C/N ratio and OUR recorded at 15 day intervals during vermicomposting.

Rea-ctors	AP (mg/kg)				C/N ratio				OUR (mg/g VS/day)			
	0 day	15 day	30 day	45 day	0 day	15 day	30 day	45 day	0 day	15 day	30 day	45 day
R1	0.40±0.05a	0.82±0.20a	1.80±0.50acde	2.20±0.20ade	49.1±0.4a	44.3±1.0a	33.9±2.1a	23.4±2.1a	18.9±2.3ab	12.5±1.3ac	8.3±1.3ae	3.4±1.1acd
CR1	0.40±0.07a	0.70±0.10a	0.82±0.05b	1.20±0.10c	49.1±0.1a	45.8±1.0a	42.6±1.0c	33.9±2.0d	18.8±1.5ab	14.5±1.0ce	12.5±1.0bcd	8.2±2.0b
R2	0.40±0.05a	1.40±0.20a	1.90±0.20acde	2.80±0.30b	29.9±3.2bd	26.4±1.1bd	20.4±2.0bde	14.2±1.5b	11.2±6.2a	8.2±2.0a	5.2±1.4a	2.4±1.0ad
CR2	0.40±0.02a	1.10±0.40a	1.70±0.30acde	1.80±0.05de	30.0±2.1bd	29.6±0.5de	26.2±0.6fg	22.1±1.2ac	11.2±5.5a	9.0±1.8a	8.6±0.6ac	7.2±0.6cb
R3	0.60±0.03ab	1.50±0.60a	2.00±0.20ade	2.50±0.20abe	29.3±0.6b	24.2±1.2bc	18.3±1.5be	12.5±0.6b	24.6±4.3b	20.9±2.1b	11.3±2.1cde	2.1±1.5a
CR3	0.60±0.02ab	1.00±0.20a	1.30±0.20abd	1.40±0.05cd	29.3±0.5b	25.2±1.5bd	22.6±1.2ef	19.0±1.5c	24.6±4.6b	21.0±3.0b	12.4±1.6bcd	6.8±1.6cb
R4	0.80±0.02b	1.18±1.00a	2.00±0.40ae	2.80±0.26b	33.8±1.2d	23.7±2.0b	18.7±2.1be	12.2±1.6b	21.4±2.5ab	15.7±1.8cb	8.7±1.5ac	2.1±1.3a
CR4	0.80±0.03b	1.08±0.20a	1.20±0.15cb	1.70±0.18acde	33.6±1.5d	28.3±2.0cd	24.4±1.3dfh	21.0±1.0ac	21.1±3.8ab	18.5±2.0be	14.0±1.4d	6.3±2.1bd
R5	0.70±0.02b	1.70±0.80a	2.20±0.22e	2.80±0.30b	43.2±0.8c	23.5±1.2b	17.7±1.5b	10.2±1.7b	10.7±3.7a	7.6±1.8a	5.4±1.0a	1.8±1.2a
CR5	0.70±0.24b	1.00±0.30a	1.70±0.20ace	2.20±0.20e	43.0±0.9c	33.0±2.0e	27.8±1.5gh	22.5±0.8ac	9.6±5.0a	8.2±0.6a	6.9±1.0a	5.2±1.0ab

Mean values followed by different letters in columns are statistically different (ANOVA; Tukey's test, $P < 0.05$)

2009b). The greatest increase in ash content was recorded in T2 (24.2%), T5 (23.1%) and T3 (20.8%) and least in CT5 (10.4%), CT4 (10.7%) and CT3 (17.6%), respectively (Table 2). Earlier workers also report an increase in ash content (Yadav and Garg 2009; Deka et al. 2011a). The variation in ash content was statistically significant ($P < 0.05$).

Total organic carbon (TOC)

There was a decrease in organic carbon in all the treatments probably due to substrate mineralization, brought about by the metabolic activity of the earthworms and associated micro flora (Orozco et al. 1996). Final TOC was lower in vermicompost and compost as compared to the initial value. At the end of bioconversion period, a significant fraction of the TOC contained in the initial mixture was lost as CO_2 (Elvira et al. 1996) possibly the result of the available carbon being used as a source of energy by the earthworms and microbes (Khwairakpam and Bhargava 2009b). The physical, biological and chemical environment of the waste is modified by the earthworms, which make the waste more suitable for colonization by microbial communities, which in turn results in loss of carbon (Suthar and Singh 2008). The greatest decrease in TOC was recorded in T5 (42.7%) and CT3 (21.1%) and least in T1 (19.4%) and CT4 (15.8%) (Table 2). These results are similar to those reported previously of between 20–43% (Elvira et al. 1997) and up to 45% (Kaviraj and Sharma 2003). Variation in TOC in the different treatments was statistically significant ($P < 0.05$).

Nitrogen content

TN increased in all the treatments, possibly a result of the activity of earthworms, as reported by other authors (Suthar and Singh 2008). Earthworms also have a great effect on the transformation of nitrogen in manure, by enhancing nitrogen mineralization, which results in nitrogen being retained in the form of nitrates (Atiyeh et al. 2000). However, in general the final nitrogen content of compost is dependent on the initial nitrogen content in the waste. TN consists of the inorganic forms of nitrogen ammonium (NH_4-N) and nitrate (NO_3-N). The greatest TN was recorded in T5 (58.7%) followed by T4 (49.7%), T3 (43.2%) and least in CT2 (8.2%) followed by CT1 (16.3%) and CT (17.9%), respectively (Table 2). Also using the same species of earthworm, earlier workers also report many fold increases in TN (Suthar and Singh 2008; Khwairakpam and Bhargava 2009a; Deka et al. 2011b). A decrease in NH_4-N and corresponding increase in NO_3-N was recorded during the final stages of the vermicomposting process (Table 3). Over the first 15 days there was a decrease in the amount of NH_4-N (98.8%). The greatest change of 98% was recorded in T1. This might be due to the loss of NH_4-N as volatile ammonia at high pH values. There was a 1.8–7.4 fold increase in NO_3-N during the later stages of the composting process. Greatest change was recorded in T1 (7.4 fold). The difference in the various forms of nitrogen is due to immobilization/denitrification or both (Khwairakpam and Bhargava 2009a). ANOVA revealed that the differences are statistically significant ($P < 0.05$).

Phosphorus turnover

Total phosphorus (TP) content was greater at the end of the composting process, probably because of mineralization of organic matter (Elvira et al. 1997). The greatest TP was recorded in T5 (76.1%) followed by T2 (74.5%) and CT5 (73.4%), and the least in CT1 (17.4%) followed by T1 (45.1%) and CT2 (59.1%), respectively (Table 4). Similar results are reported by other workers who record the stimulating effect of earthworms on the availability of phosphorous in soil (Krishnamoorthy 1990; Kaviraj and Sharma 2003; Tognetti et al. 2005). Enzymes in the guts of earthworms have a stimulating effect on phosphate solubilizing bacteria (Satchell and Martin 1984). Available phosphorus (AP) is more important for plant maturation than plant growth (Yadav and Garg 2009). Addition of phosphorus to vermicompost also prevents the loss of nitrogen by the volatilization of ammonia (Yadav and Garg 2011). Vermicomposting is an efficient way of transforming unavailable forms of phosphorous into forms easily available to plants (Ghosh et al. 1999). Similar increases in AP were recorded in the vermicompost, with greatest increase recorded in T2 (85.7%) (Table 5). The variation in TP was significant ($P < 0.05$).

C/N ratio

The C/N ratio is the most reliable indicator of the degree of decomposition and whether the compost is ready for field application. In general, the carbon content decreased and that of nitrogen increased during the decomposition process in all treatments. However, the C/N ratio varies widely depending on the rate of decomposition (Subramanian et al. 2010). The final values of the C/N ratio in all the containers were in the range of 10-23, which indicates an advanced degree of organic matter stabilization and a satisfactory degree of maturity of the organic waste (Senesi 1989) (Table 5). The greatest change was recorded in T5 (4.2 fold) followed by T4 (2.7 fold) and T3 (2.3 fold) and least in CT2 (1.3 fold) followed by CT1 (1.4 fold) and CT3 (1.5 fold), respectively. Earthworms digest long chain polysaccharides and enhance the colonization of the compost by microbes (Aira et al. 2007), which further accelerates the rate of organic matter degradation and nitrogen fixation (Garg and Kaushik 2005). This results in a greater decline in C/N ratio in the vermicompost than in the compost. Some nitrogen is also added by the worms during vermicomposting in the form of mucus, nitrogenous excretory substances, hormones and enzymes (Hobson et al. 2005; Neuhauser et al. 1986). The decrease in the C/N ratio recorded over time might also be attributed to the increase in the earthworm population (Ndegwa and Thompson 2000). Earthworms decompose the carbonaceous wastes efficiently and lower the C/N ratio, which might account for lower C/N ratio recorded in vermicompost than in compost. C/N ratio varied significantly in all vermicompost and compost treatments ($P < 0.05$).

Rate of oxygen uptake (OUR)

Initially the OUR recorded in all the treatments was high possibly due to the rapid growth of microbes. OUR is the most frequently used method for determining the biological activity of waste material (Gomez 2006). It measures compost stability by evaluating the amount of readily biodegradable organic matter still present in a sample based on the oxygen demand (Khwairakpam and Bhargava 2009b). The OUR was greatest during the active stage of composting, as microbes grow rapidly because there is an abundance of readily biodegradable substrate (Kalamdhad and Kazmi 2009). At the onset of the composting process large organic molecules are broken down into smaller, soluble ones and temporarily more substrate may become available. The decrease in OUR was greatest in T3 (11.3 fold) followed by T4 (10.1 fold) and T1 (5.5 fold) and least in CT2 (1.5 fold) followed by CT5 (1.8 fold) and CT1 (2.2 fold) (Table 5). Earlier researchers also record a decrease in OUR during the vermicomposting process (Khwairakpam and Bhargava 2009b). The changes in OUR were statistically significant ($P < 0.05$).

Rate of production of CO_2

The rate of production of CO_2 was greatest during the initial stages of the composting process possibly due to a high level of microbial activity. The production of CO_2 is the most direct way of determining the stability of the compost because it measures carbon that originates directly from the compost. Thus, the production of CO_2 directly correlates with aerobic respiration, which is the best measure of aerobic biological activity (Khwairakpam and Bhargava 2009b). The greatest decrease in respiratory activity was recorded during the initial stages of composting. The greatest production of CO_2 was recorded in T5 (6.7 fold) followed by T3 (5.6 fold) and T2 (4.8 fold), and least in CT1 (1.9 folds) followed by CT2 (2.7 fold) and CT4 (2.4 fold) (Table 6). Waste in the early stages of composting has a strong demand for O_2 and produces large quantities of CO_2 due to a great increase in the numbers of micro-organisms that develop on the abundance of easily biodegradable compounds in the raw material. For this reason, O_2 consumption or CO_2 production can be used as an indicator of the stability and maturity of the compost (Kalamdhad et al. 2008). All the variations in CO_2 production were significant ($P < 0.05$).

Table 6 CO_2 evolution, soluble BOD and COD recorded at 15 day intervals during vermicomposting.

Reactors	CO_2 evolution (mg/g VS/day)				Soluble BOD (mg/kg)				Soluble COD (mg/kg)			
	0 day	15 day	30 day	45 day	0 day	15 day	30 day	45 day	0 day	15 day	30 day	45 day
R1	8.75±1.20abc	5.75±1.10ab	3.24±1.00a	2.88±0.60abc	30.8±2.0a	24.3±2.2a	12.4±2.4abc	5.5±1.5a	591±30a	455±12a	250±50a	150±20a
CR1	8.75±1.20abc	7.44±0.50ab	5.45±1.20a	4.55±0.20b	30.8±1.1a	25.3±1.4a	20.2±1.6b	17.2±1.5b	591±45a	512±30a	368±20a	260±40a
R2	12.24±1.10a	8.56±1.50ab	4.92±2.10a	2.50±1.18abc	32.8±4.0a	23.0±4.0a	10.5±2.0ac	4.7±3.2a	16631±250c	11254±120c	645±150ab	307±35a
CR2	12.28±1.20ac	9.36±2.00b	6.52±1.60a	4.50±1.25cb	32.6±3.0a	24.6±4.2a	18.6±3.0cb	15.5±1.6b	16675±100c	12756±220e	7486±180d	3004±150c
R3	12.18±1.50a	8.32±2.40ab	3.24±1.50a	2.17±0.30abc	32.1±2.5a	22.4±2.5a	10.0±2.6a	3.4±1.5a	15800±200d	5621±300b	480±105ab	142±32a
CR3	12.22±1.30ac	9.26±2.30ab	5.44±2.00a	3.48±1.00abc	28.4±6.5a	23.5±2.0a	17.1±2.3abc	14.3±1.0b	15196±255f	7745±310d	5007±300c	984±261b
R4	8.62±1.50abc	5.32±0.60ab	3.54±1.00a	2.15±0.60ac	34.0±5.0a	22.0±5.0a	10.0±5.0a	4.5±2.3a	14620±321e	5442±210b	814±215b	217±125a
CR4	8.57±1.00abc	6.67±0.20ab	4.56±1.20a	3.44±0.25abc	33.7±3.3a	25.5±4.3a	17.1±3.0abc	12.6±1.5b	14901±100e	7214±300d	4897±240c	995±130b
R5	8.15±2.00b	5.01±1.10a	3.52±1.10a	1.20±1.00a	34.4±1.8a	21.5±2.6a	9.6±2.6a	3.2±1.0a	13512±246b	5289±250b	725±120a	140±41a
CR5	8.19±1.00b	5.22±1.20ab	3.80±1.00a	2.84±1.00abc	36.7±2.1a	25.0±3.1a	16.4±2.4abc	12.3±1.1b	13822±233b	7077±300d	4352±213e	885±124b

Mean values followed by different letters in columns are statistically different (ANOVA; Tukey's test, $P < 0.05$)

Soluble BOD and COD

It is generally recognized that the percentage of readily biodegradable organic matter is an important determinant of compost quality. Only the C/N ratio gives a clear indication of the stability of the compost because O_2 consumption continues, which indicates the compost is still immature. In order to determine the O_2 consumption it is necessary to measure soluble BOD and COD. When applying compost to soil for crop use, care should be taken because the biological processes will continue and can strip nutrients from soil even if the compost is stable (Wang et al. 2004). Hence it is important to monitor BOD. The decrease in BOD was greatest in T5 (10.7 fold) followed by T3 (9.3 fold) and T4 (7.5 fold), and least in CT1 (1.7 fold), CT3 (1.9 fold) and CT2 (2.1 fold) (Table 6). The greatest reduction in COD was recorded in T3 (111.2 fold), T5 (96.5 fold) and T4 (67.3 fold), and the least in CT1 (2.2 fold), T1 (3.9 fold) and CT2 (5.5 fold), respectively. The variations in BOD and COD at the end of the composting process were significant ($P < 0.05$) in all the treatments.

Growth and reproduction of earthworms

After 45 days of earthworm activity the vermicompost was a dark brown colour and had an homogenous texture. The earthworm biomass increased in all the vermicomposts. Earthworm biomass increased in all the treatments except for T1, where there was a 5.5% reduction in biomass (Table 7). This may be due to the nature of substrate, which consisted of 100% dewatered sludge. The greatest increase in earthworm biomass was recorded in T5 (19.3%) followed by T4 (16.6%) and T3 (10.7%), respectively. Cocoon production was greatest in T3 with 0.017 cocoons/worm/day followed by T2 (0.015 cocoons/worm/day) and T4 (0.014 cocoons/worm/day). Other researchers working with *P. excavatus* also report an increase in cocoons/worm/day (Knieriemen 1985; Reinecke et al. 1992; Suthar 2006; Khwairakpam and Bhargava 2009b; Deka et al. 2011b). The number of juveniles that hatched per 100 gm of end product was quite high, with the greatest number of 90 recorded in T5 and least in T2 (28).

Conclusions

The end product after 45 days of vermicomposting was a dark brown colour and smelt of humus. *P. excavatus* effectively processed the organic wastes and produced an end product rich in TN and TP. The higher nutrient content, lower C/N ratio and EC, plus higher pH of the vermicompost indicates that vermicomposting is the better option for disposing of dewatered sludge from pulp and paper mills. Overall T5 proved to be the best combination for vermicomposting followed by T3, T2, T4 and T1, respectively. The low biomass of earthworms recorded in T1 may be due to the nature of the substrate, which consisted of 100% DS. However, the growth of earthworms was good in all the other treatments and best in T5. The results indicate that there is an inverse relationship between the growth rate and cocoon production by earthworms and the percentage of DS in the waste material.

Acknowledgements

The authors are very thankful to Dr. Nikhileshwar Sharma, Senior Manager, Nagaon Paper Mill, Kagajnagar for providing the dewatered sludge.

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Table 7 Live biomass production of earthworms in different treatments.

Vermireactor	Mean weight of Earthworms (g)		Live biomass (% change)	Coocons/worm/day	No. of Juveniles hatched/100 g
	Initial	Final			
T1	50	47±1.6a	–5.6±3.6ab	0.004±0.001ae	30±1a
T2	50	55±1bc	10±1.7ab	0.015±0.005bc	28±2b
T3	50	56±3cd	12±6bc	0.017±0.002c	50±1cd
T4	50	60±2de	20±4cd	0.014±0.003db	55±3d
T5	50	62±1.5e	24±3d	0.009±0.001ebd	90±4e

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BIOASSOCIATIVE EFFECT OF RHIZOSPHERIC MICROORGANISMS ON GROWTH, NUTRIENT UPTAKE AND YIELD OF MUNG BEAN (*VIGNA RADIATA* L. WILCZEK)

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ABSTRACT

Nitrogen applications have generated great interests in agriculture, with much of its success associated with increasing the uptake of nitrogen by crops while reducing pollution by this chemical fertilizer. The aim of the present study was to evaluate the interactive effect of rhizospheric microorganisms on nutrient uptake, yield and growth of mung bean grown in pots under glasshouse conditions. The results revealed that the growth, in terms of morphology and physiology, of all the inoculated plants was better than that of the control plants. In terms of growth, plant height, fresh and dry weights and length of the roots plants inoculated with both *Funneliformis mosseae* + *T. viride* did best. Total chlorophyll content, alkaline and acidic phosphatase activities were greatest when inoculated with only *F. mosseae* and fresh and dry weights of shoots when inoculated only with *T. viride*. Significant increase in N and P uptake was recorded when inoculated with both *F. mosseae* + *T. viride*. Overall the significant increase in growth and development was due to positive interactions among rhizospheric microorganisms leading to healthy and vigorously growing plants. However, there is now a need for field trails of this technique.

Keywords: Arbuscular mycorrhizal fungi, nutrient uptake, phosphatase activity, nitrogen fixing bacteria, *Trichoderma viride*, mung bean

Introduction

Legumes are important land plants as they are able to grow in water-deficient and low-nutrient environments. Many legumes form symbiosis with both nitrogen fixing rhizobia and arbuscular mycorrhizal (AM) fungi. Dual inoculation with both microorganisms results in a tripartite mutualistic symbiosis and increases plant growth to a greater extent than inoculation with only one of them (Chalk et al. 2006). *Vigna radiata* (L.) Wilczek commonly known as mung bean is an important grain legume crop in South East Asia and Africa, and a source food that has a high nutritive value (Kumar et al. 2002; Salunke et al. 2005). It is not only a rich and economical source of protein, phosphorus, carbohydrate, minerals and provitamin A, but also commonly used as fodder and green manure. Mung bean contains bioactive components with antioxidant, antimicrobial and insecticidal properties (Bounce 2002; Kaprelynts et al. 2003; Madhujith et al. 2004; Ahmad et al. 2008).

The scarcity of food containing high levels of protein, micronutrients and various vitamins sources is an increasing problem affecting millions of people in developing countries (Burchi et al. 2011). The need for innovative technologies to meet the growing challenges of scarcity of food and malnutrition in the poor and hungry parts of the world must be addressed (Clugston 2002). To improve the quality and yield of economically important legumes, particularly mung bean, farmers apply large quantities of chemical fertilizer, which has detrimental effects on the soil, such as the accumulation of toxic salts. Application of environmentally friendly and potentially cost effective microbial bio-fertilizer could be a better solution (Mia and Shamsuddin 2010).

Structural and physiological studies have revealed that legumes form tripartite symbiotic associations with nodule forming rhizobia and AM fungi (Barea et al. 2005). The micro-symbionts in both associations are benefited by photo assimilates from the plant. The macro-symbionts fix nitrogen in the case of the bacterial symbiosis in root nodules (Brewin 1991; Crespi and Galvez 2000) and immobile nutrients, especially phosphate, in the case of AM symbiosis (Stract et al. 2003; Miransari et al. 2009). Nitrogen availability and P uptake from less soluble sources is an important issue, thus it is important to understand the tripartite association of legumes with *Rhizobium* and AM (Kwapata and Hall 1985; Satter et al. 2006). There is increasing evidence that diverse microbial populations in the rhizosphere play a significant role in agricultural sustainability (Barea et al. 2002). However, due to the obligatory biotrophy of AM fungi, the two symbioses are rarely studied together. Moreover, the initiation and functioning of the tripartite symbiosis is complex and despite efforts made over the last two decades or so, there are still many facts about this symbiosis that are unknown (Gianinazzi-Pearson et al. 1995). Considering the beneficial effect of AMF on other crops, more attention should be paid to combining appropriate mycorrhizal fungi with other soil microbes in order to increase yields of mung bean. With this in mind, a pot experiment was carried out under glass house conditions to investigate the effect of AM fungi alone or in combination with other bio-inoculants on mung bean.

Material and methods

Mass multiplication of bio-inoculants

The two dominant AM species used in this study were *Funneliformis mosseae* (T.H. Nicolson and Gerd.) Walker and Schüβler and *Acaulospora laevis* Gerdemann and Trappe. These species were isolated from the rhizosphere of mung beans collected from the Botanical Garden, Kurukshetra University, Kurukshetra and kept in sterilized polythene bags at 10 °C for further processing**.** The AM species were propagated in association with barley under standard pot culture conditions. The culture of *Rhizobium* sp. (procured from Department of Microbiology, CCS Haryana Agricultural University, India) was mass multiplied using nutrient broth medium and that of *T. viride* on a modified wheat bran-saw dust medium (Mukhopadhyay et al. 1986).

Preparation of pot mixture

The experiment was laid out in a randomized complete block design, with five replicates of each treatment. Top soil (0–30 cm) was collected from the Botanical garden of Botany Department, Kurukshetra University, Kurukshetra, which consisted of 20.8% silt, 3.78% clay, and had a pH of 8.05, 0.0485 total N and 0.015% available P. This soil was air dried and passed through a 2 mm sieve. The soil to be used in the experiment consisted of a soil: sand mixture (3:1, v/v), which had been autoclaved for 20 minutes at 121 °C and 15psi. Each pot was filled with the sterilized soil:sand mixture to which 10% (w/w of soil) of the selected AM inoculum was added. *T. viride* inoculum containing 3.4×10^8 cfu gm^{-1} was added as per the treatment. The seeds were surface sterilized with 10% solution of sodium hypochlorite for 1–2 minutes and then washed thoroughly with distilled water. Before sowing seeds 10 ml of a liquid suspension of bacteria, with a density 10^8 cells/ml, was applied to each pot. Pots were watered once every two days, and fertilized with a nutrient solution after 15 days (Weaver and Fredrick 1982), which contained half the recommended level of phosphorus and no nitrogen. Pots were either treated with a single inoculum, a combined inoculum or no inoculum as outlined below:

1. Uninoculated i.e. autoclaved sterile sand: soil with no inoculum (control 1)
2. Uninoculated i.e. unsterilized sand: soil with no inoculum (control 2)
3. *Funneliformis mosseae* (F)
4. *Acaulospora laevis* (A)
5. *Trichoderma viride* (T)
6. F + T
7. A + T
8. F + A
9. F + A + T

Table 1 Combined effect of AM fungi, *Rhizobium* and T. *viride* on the growth of mung bean 120 days after inoculation.

Treatments	Plant height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	AM spore number / 10 g of soil	Root colonization (%)	Number of nodules / plant
C1	14.5 ± 1.25^{f}	1.18 ± 0.05^{g}	0.43 ± 0.003^{g}	7.1 ± 0.55^{g}	0.60 ± 0.004^{f}	0.16 ± 0.006^{f}	19.8 ± 2.86^{g}	20.6 ± 2.40^{h}	1.4 ± 0.54^{f}
C2	19.3 ± 1.78^{e}	3.01 ± 0.05^{f}	0.59 ± 0.004^{f}	8.3 ± 0.40^{f}	0.62 ± 0.004^{f}	0.20 ± 0.003^{ef}	49.0 ± 2.64^{f}	32.8 ± 1.92^{g}	4.2 ± 0.83^{e}
F	30.2 ± 2.38^{b}	5.81 ± 0.12^{c}	1.97 ± 0.008^{c}	18.8 ± 1.92^{b}	0.97 ± 0.041^{b}	0.41 ± 0.014^{b}	109 ± 2.54^{b}	79.2 ± 1.92^{b}	8.6 ± 0.89^{bc}
A	20.6 ± 2.70^{de}	3.13 ± 0.01^{f}	0.62 ± 0.003^{f}	9.8 ± 2.58^{e}	0.72 ± 0.006^{e}	0.29 ± 0.016^{d}	81.2 ± 3.11^{d}	53.8 ± 2.70^{d}	6.0 ± 1.22^{d}
T	25.8 ± 2.38^{bc}	8.54 ± 0.05^{a}	3.47 ± 0.014^{a}	14.6 ± 2.40^{c}	0.92 ± 0.005^{c}	0.40 ± 0.008^{b}	$52.0 \pm 1.58e^{f}$	37.4 ± 2.07^{ef}	6.4 ± 1.51^{d}
F + T	40.6 ± 2.40^{a}	7.02 ± 0.01^{b}	2.97 ± 0.009^{b}	22.6 ± 2.40^{a}	1.55 ± 0.060^{a}	1.05 ± 0.090^{a}	80.2 ± 2.38^{d}	55.6 ± 2.70^{d}	9.8 ± 1.48^{a}
A + T	24.0 ± 1.58^{c}	4.74 ± 0.01^{d}	0.89 ± 0.003^{d}	15.0 ± 1.58^{c}	0.85 ± 0.005^{d}	0.36 ± 0.020^{c}	56.4 ± 1.94^{e}	42.4 ± 1.81^{e}	7.2 ± 1.78^{c}
F + A	22.8 ± 1.92^{cd}	4.12 ± 0.01^{e}	0.80 ± 0.008^{e}	10.6 ± 0.44^{d}	0.69 ± 0.003^{e}	0.23 ± 0.021^{e}	119.2 ± 2.16^{a}	89.8 ± 1.64^{a}	7.6 ± 0.54^{c}
F + A + T	28.2 ± 2.38^{b}	6.98 ± 0.01^{b}	2.14 ± 0.044^{bc}	17.4 ± 2.70^{b}	0.87 ± 0.002^{c}	0.32 ± 0.023^{cd}	94.8 ± 2.38^{c}	62.2 ± 2.38^{c}	9.4 ± 1.34^{ab}
L.S.D ($P \le 0.05$)	3.0241***	0.5075***	0.57595***	3.1551***	0.0506***	0.0505***	6.4936***	5.3536***	1.6826***
ANOVA (F7,16)	45.098	156.879	26.920	21.802	262.79	228.58	228.588	138.32	19.820

Control1 (C1) – Un-inoculated sterile soil; Control 2 (C2) – Un-inoculated soil; F – *Funniliformis mosseae;* A – *Acaulospora laevis;* T – *Trichoderma viride;* F + T – *Funniliformis mosseae* + *Trichoderma viride;* A + T – *Acaulospora laevis* + *Trichoderma viride;* F + A – *Funniliformis mosseae* + *Acaulospora laevis;* F + A + T – *Funniliformis mosseae* + *Acaulospora laevis* + *Trichoderma viride.*
Values are means ± standard error, *** significant values.
Means were compared using the least significant difference (LSD) test ($P \le 0.05$). Data in each column followed by dissimilar letters differ significantly at $P \le 0.05$.

Table 2 Combined effect of AM fungi, *Rhizobium* and *T. viride* on physiological features of mung bean 120 days after inoculation.

Treatments	Chl. content (mg/100mg FW)		Total Chl.	Phosphatase activity (IUg^{-1}FW)		Stomatal conductance (mmol^{-2} s^{-2})		No. of pods	Wt. of pods (gm)
	Chl a Chl b			Acidic alkaline		Morning evening			
C1	0.29 ± 0.01^{g}	0.23 ± 0.02^{f}	0.53 ± 0.04^{g}	0.078 ± 0.019^{f}	0.004 ± 0.002^{d}	L 115.70 ± 1.63^{h} U 19.47 ± 1.40^{f}	L 91.46 ± 1.41^{h} U 12.50 ± 1.52^{e}	4.6 ± 1.14^{g}	0.92 ± 0.01^{h}
C2	0.47 ± 0.03^{f}	0.33 ± 0.02^{e}	0.81 ± 0.02^{f}	0.098 ± 0.03^{f}	0.006 ± 0.003^{d}	L 234.81 ± 3.61^{h} U 23.04 ± 2.06^{e}	L 95.48 ± 2.59^{g} U 13.34 ± 0.55^{e}	7.8 ± 0.83^{f}	1.06 ± 0.006^{g}
F	1.04 ± 0.03^{b}	0.53 ± 0.03^{bc}	1.59 ± 0.03^{b}	0.270 ± 0.025^{a}	0.202 ± 0.028^{a}	L 453.54 ± 2.19^{a} U 54.78 ± 1.95^{b}	L 286.05 ± 3.13^{c} U 20.43 ± 1.54^{b}	14.0 ± 2.23^{c}	4.95 ± 0.03^{d}
A	0.64 ± 0.02^{e}	0.48 ± 0.02^{c}	1.12 ± 0.04^{e}	0.148 ± 0.025^{e}	0.008 ± 0.001^{d}	L 310.96 ± 3.11^{d} U 21.21 ± 1.70^{ef}	L 256.37 ± 2.56^{d} U 16.41 ± 1.39^{cd}	10.4 ± 1.14^{d}	3.94 ± 0.02^{e}
T	0.75 ± 0.03^{d}	0.42 ± 0.07^{d}	1.18 ± 0.07^{de}	0.216 ± 0.024^{cd}	0.146 ± 0.020^{c}	L 258.49 ± 2.81^{f} U 64.26 ± 1.59^{a}	L 107.74 ± 2.09^{f} U 15.52 ± 1.30^{d}	22.0 ± 2.54^{ab}	6.28 ± 0.04^{b}
F+T	0.83 ± 0.06^{c}	0.56 ± 0.07^{b}	1.40 ± 0.09^{c}	0.250 ± 0.022^{ab}	0.180 ± 0.015^{b}	L 340.84 ± 1.92^{c} U 31.54 ± 1.54^{c}	L 317.98 ± 2.05^{a} U 24.53 ± 1.64^{a}	24.2 ± 1.92^{a}	7.80 ± 0.01^{a}
A+T	0.74 ± 0.03^{de}	0.37 ± 0.02^{e}	1.17 ± 0.12^{de}	0.154 ± 0.020^{e}	0.009 ± 0.001^{d}	L 303.36 ± 2.82^{e} U 32.71 ± 1.95^{c}	L 245.51 ± 2.49^{e} U 18.09 ± 1.26^{c}	9.4 ± 1.14^{ef}	2.97 ± 0.02^{g}
F+A	0.63 ± 0.02^{e}	0.58 ± 0.02^{b}	1.21 ± 0.03^{d}	0.230 ± 0.015^{bc}	0.164 ± 0.020^{bc}	L 238.54 ± 2.89^{g} U 26.31 ± 1.48^{d}	L 105.36 ± 2.92^{f} U 17.02 ± 2.02^{cd}	10.0 ± 1.58^{de}	3.70 ± 0.01^{f}
F+A+T	1.16 ± 0.02^{a}	0.70 ± 0.01^{a}	1.86 ± 0.02^{a}	0.194 ± 0.024^{d}	0.154 ± 0.020^{c}	L 371.68 ± 2.86^{b} U 32.03 ± 1.89^{c}	L 303.60 ± 2.24^{b} U 17.28 ± 1.45^{cd}	21.4 ± 2.40^{b}	5.83 ± 0.03^{c}
L.S.D(P<0.05)	0.0359***	0.0523***	0.0808***	0.0301***	0.0209***	L 3. 4914*** U 2 .2438***	L 3.1317*** U 1.8747***	2.2665***	0.0356***
ANOVA (F 7,16)	356.04	61.169	194.441	40.664	142.886	L6211.873 U 390.647	L 8122.36 U 30.842	79.907	34809.964

Control1 (C1) - Un-inoculated sterile soil; Control 2 (C2) - Un-inoculated soil; F- *Funniliformis mosseae*; A - *Acaulospora laevis*; T - *Trichoderma viride*; F+T - *Funniliformis mosseae + Trichoderma viride*; A+T - *Acaulospora laevis +Trichoderma viride*; F+A - *Funniliformis mosseae + Acaulospora laevis*; F+A+T – *Funniliformis mosseae + Acaulospora laevis + Trichoderma viride.*

Values are means ± standard error, *** significant values.

Means were compared using the least significant difference (LSD) test ($P \leq 0.05$). Data in each column followed by dissimilar letters differ significantly at $P \leq 0.05$.

Plant harvest, growth and nutrient analysis

Plants were harvested after 120 days by uprooting them from the soil and various morphological and physiological features measured. For determining root and shoot fresh and dry weight, roots and shoots were harvested after 120 days, weighed and then, oven dried at 70 °C and weighed again. Amount of chlorophyll a, chlorophyll b and total chlorophyll was estimated using the method of Arnon (1949). Stomatal conductance ($mmol^{-2} sec^{-2}$) was measured using a Porometer (AP_4-DELTA-T DEVICES-Cambridge-U.K.). Phosphorus concentration were determined using the 'Vanado-molybdo-phosphoric yellow colour method' (Jackson 1973) and Nitrogen using the Kjeldahl method. Phosphatase activity was assayed using p-nitrophenyl phosphate (PNPP) as a substrate, which is hydrolyzed by the enzyme to p-nitrophenol (Tabatabi and Bremner 1969).

Identification and quantification of the number and colonization by AM spores

AM spores (*F. mosseae* and *A. laevis*) were identified by using the identification manual used by Walker (1983), Scheneck and Perez (1990), Morton and Benny (1990) and Mukerji (1996). Quantification of the number of AM spores was done using the Adholeya and Gaur 'Grid Line Intersect Method' (1994). Mycorrhizal colonization of roots was determined using the 'Rapid Clearing and Staining Method' of Phillips and Hayman (1970). Percentage AM colonization of roots was: (Number of root segments colonized / number of root segments studied) × 100.

Statistical analysis

Data were subjected to analysis of variance and means separated using the least significant difference test in the Statistical Package for Social Sciences (ver.11.5, Chicago, IL, USA).

Results

It is evident from (Table 1) that inoculated plants were taller than the un-inoculated control plants. The increase in plant height was significantly greatest for those inoculated with both *F. mosseae* + *T. viride* followed by those inoculated only with *F. mosseae*. This indicates that mung bean benefited from the particular mycorrhizal symbioses used in this study.

Maximum increment in shoot biomass was recorded in single inoculation treatment with *T. viride* followed by dual inoculation with *F. mosseae* and *T. viride*. According to the results in Table 1 root biomass (fresh and dry) was also significantly greater in all the treatments than the control. After 120 days, the increase in root biomass was greatest in the dual treatment with *F. mosseae* and *T. viride* followed by that in the single treatment with *F. mosseae*.

The greatest root length was recorded for plants inoculated with both *F. mosseae* and *T. viride* followed by those inoculated only with *F. mosseae*.

The result indicates that the mycorrhizal colonization of roots and number of spores of AM recorded for the treated plants varied (Table 1). The maximum number of spores and percentage colonization of roots was recorded for plants treated with *F. mosseae* + *A. laevis* and *T. viride* and then those treated with a single inoculation of *F. mosseae*.

Chlorophyll content of all the treated plants was greater than that of the control. According to Table 2 the highest increment in total chlorophyll content was recorded for plants treated with *F. mosseae* + *A. laevis* + *T. viride* and then those treated with a single inoculation of *G. mosseae*.

According to Table 2 the phosphatase activity recorded for inoculated plants was considerably greater than that recorded for the control. This is due to the mycorrhizal fungal hyphae that secrete acid and alkaline phosphatases (APA and ALA) into the rhizosphere. The acidic phosphatase activity was greatest for the *F. mosseae* treated plants followed by those treated with *F. mosseae* + *T. viride.* In addition, the greatest alkaline activity was recorded for the treatment with *F. mosseae*. Acidic phosphatase activity was greater than that of alkaline phosphatase.

Mycorrhizal colonization of roots also influences the behaviour of the stomata of the leaves. Stomatal conductance (morning and evening) of plants treated with different combinations of AM fungi and *T. viride* increased (Table 2). Stomatal conductance was greatest in the morning in plants treated with *F. mosseae* and in the evening in those treated with *F. mosseae* + *T. viride*. The conductance recorded for the stomata on the lower surface of leaves was greater in the morning than the evening.

The maximum number of nodules was recorded for the dual inoculation treatment, i.e. with *F. mosseae* and *T. viride,* followed by the triple inoculation treatment with *F. mosseae*, *A. laevis* and *T. viride*. The inoculation of several different microbes appears to result in a synergistic interaction among them that substantially improves the nutrition available to the mung bean plants.

AM fungi improved the mineral nutrition of associated plants (Table 3). Mung bean plants inoculated with AM fungi and *T. viride* had increased N and P contents in roots and shoots compared with the control. Among all the treated plants those inoculated with *F. mosseae* showed the greatest percentage increase in shoot and root P content and those treated with both *F. mosseae* + *A. laevis* the greatest percentage in shoot and root N. This might be due to an increase in carbon supplied by the plant to the AM fungi, which increases the uptake and transfer of nutrients from fungi to plants. In the present study, more P than N was absorbed by all the AM inoculated mung bean plants. The increase in P content was due to the AM fungal network that can take up P and convert it into polyphosphate granules in the extra radical hyphae and pass it to the arbuscules, which then make it available to plants. The increase in total N is attributed to the increased N_2 fixation by the AM hyphae.

Data on yield (Table 2) indicate that bioinoculation of mung bean plants with either AM or *T. viride* resulted in a significant increase in the weight of the pods and consequently higher yields compared to the control. Treatment with a mixture of *F. mosseae* and *T. viride* resulted in the greatest increase in yield in terms of the number of pods followed by those plants treated with *T. viride.* That is, the greatest yield was of plants inoculated with both AM fungi and *T. viride,* which operated together more effectively in supplying their host plants with their nutrient requirements than when either operated on its own.

Discussion

In the present investigation, dual inoculation or a mixed inoculation gave better results than either of the two inoculates on their own. The F + T treatment gave better results than the other treatments because *Trichoderma* might produce extra cellular metabolites that enhance the AM population in the soil. The increased height of mycorrhizal plants is attributed to a higher AM colonization, which is known to enhance plant growth by increasing nutrient uptake and plant growth promoting substances in the rhizosphere of mung bean. The beneficial effects of AM symbiosis are also known to be mediated by phytohormones,, which could be suitable candidates for signaling between plants and AM fungi and important in the auto regulation of mycorrhization (Meixner et al. 2005). These observations accord with earlier reports of Nzanza et al. (2011) that *Solanum lycopersicum* inoculated with *Trichoderma harzianum* and AM fungi are taller than control plants. Vázquez et al. (2000) also found that inoculation with AM and other microbes resulted in taller maize plants. The better results obtained when unsterilized rather than sterilized soil is used may be due to the presence of natural micro-flora in the soil. This may be due to competition for space and nutrition between the diverse microflora in unsterilized soil.

Khan et al. (2008) also report a significant increase in shoot and root dry weight of *Medicago sativa* following inoculation with AM fungi. Similar results are also reported by Arumugam et al. (2010) for *Vigna unguiculata* inoculated with AM and *Rhizobium*. This may be due to the fact that there were no indigenous microbes to compete with the inoculated strain. Secondly, the greater reproduction of AM fungi in soil based inoculums, which sprouted rapidly from extracellular and intracellular hyphae, absorbsed more nutrients, even far from the surface of the roots, which enhanced the growth and biomass of the plants.

The better results obtained when only *F. mosseae* was inoculated may be due to better space and nutrition for its multiplication and survival in sterilized soil, and therefore it was able to absorb more nutrients from the soil resulting a better growth of mung bean. In addition, the mycorrhizal colonization of the soil in effect enabled the plants' root systems to extend deeper into the soil and invade nutrient depleted zones and enhance soil aggregation, which ultimately improves the access of the root system to soil water and nutrients. Galleguillos et

Table 3 Combined effect of AM fungi, *Rhizobium* and T. *viride* on the nitrogen and phosphorus content of mung bean 120 days after inoculation.

Treatments	Phosphorus content (%)		Nitrogen content (%)	
	Shoot Root		Shoot Root	
C1	0.05 ± 0.024^{f}	0.21 ± 0.022^{e}	1.03 ± 0.022^{g}	0.40 ± 0.024^{f}
C2	0.16 ± 0.01^{e}	0.22 ± 0.01^{de}	1.34 ± 0.025^{f}	0.51 ± 0.016^{e}
F	0.30 ± 0.028^{a}	0.36 ± 0.024^{a}	1.62 ± 0.022^{a}	0.67 ± 0.020^{b}
A	0.22 ± 0.023^{c}	0.27 ± 0.017^{c}	1.41 ± 0.028^{de}	0.54 ± 0.023^{d}
T	0.21 ± 0.016^{c}	0.25 ± 0.025^{cd}	1.40 ± 0.015^{e}	0.53 ± 0.028^{de}
F + T	0.29 ± 0.019^{ab}	0.35 ± 0.028^{ab}	1.55 ± 0.019^{b}	0.64 ± 0.020^{b}
A + T	0.19 ± 0.019^{d}	0.23 ± 0.020d^{e}	1.43 ± 0.023^{d}	0.56 ± 0.020^{cd}
F + A	0.26 ± 0.031^{b}	0.33 ± 0.022^{b}	1.64 ± 0.027^{a}	0.74 ± 0.019^{a}
F + A + T	0.20 ±0.024^{d}	0.32 ± 0.020^{b}	1.52 ± 0.031^{c}	0.58 ± 0.020^{c}
L.S.D ($P < 0.05$)	0.0317***	0.0295***	0.0314***	0.0282***
ANOVA ($F_{7,16}$)	41.182	32.305	282.229	102.191

Control1 (C1) – Un-inoculated sterile soil; Control 2 (C2) – Un-inoculated soil; F – *Funniliformis mosseae*; A – *Acaulospora laevis*; T – *Trichoderma viride*; F + T – *Funniliformis mosseae + Trichoderma viride*; A + T – *Acaulospora laevis + Trichoderma viride*; F + A – *Funniliformis mosseae + Acaulospora laevis*; F + A + T – *Funniliformis mosseae + Acaulospora laevis + Trichoderma viride.*
Values are means ± standard error, *** significant values.
Means were compared using the least significant difference (LSD) test ($P \leq 0.05$). Data in each column followed by dissimilar letters differ significantly at $P \leq 0.05$.

al. (2000) record a significant increase in root length of plants inoculated with *G. mosseae* and Chen et al. (2006) that of *Trifolium repens* and *Coreopsis drummondii* growing in soil inoculated with an isolate of *G. mosseae*.

The mixed inoculum of F + A + T gave better results than the control, which may be due to their synergistic effect. Since the spatial distribution of soil hyphae can vary among fungal species, an increase in the overall hyphal density of AM mycelium around the roots resulting from colonization by several AM fungi may be greater than colonizations by a single species. Alternately, different AM fungi may produce different amounts of soil binding agents and the interactions of these agents within the aggregates may result in a synergistic effect on soil stability. *Rhizobium* species are also effective root colonizers as they produce secondary metabolites that enhance AMF growth and thus increase mycorrhizal spore number and colonization of mung bean. In addition, *T. viride* may behave as a mycorrhizal helper by promoting higher percentage colonization and AM spore number. The results of the present investigation are in agreement with the findings of Hemashenpagam and Selvaraj (2011) as *Solanum viarum* was also inoculated with AM fungus and plant growth promoting rhizobacteria (PGPR's).

The increase in chlorophyll content recorded in the present investigation may be due to an increase in stomatal conductance, photosynthesis, transpiration and enhanced plant growth. Increase in chlorophyll content in mycorrhizal treated plants indicates an increase in the rate of photosynthesis, which can be ascribed to an increase in the absorption of nutrients. This supports the hypothesis that soil micro-organisms produce phytohormones and a number of extra-cellular enzymes, which decompose the complex organic matter before it is absorbed as a source of energy, which stimulate plant growth (Richard et al. 2007). Several workers report more chlorophyll in the leaves of mycorrhizal plants than those of non-mycorrhizal plants (Karthikeyan et al. 2009; Arumugam et al. 2010).

The activity of acid phosphatase may be associated with the growth and development of the fungus within the tissue of the host and with phosphorus acquisition by the rhizosphere. In the present investigation, it was found that phosphatase activity (alkaline and acidic) in plants was greatest in those with the greatest mycorrhizal root colonization. Alkaline phosphatase activity is also closely linked with both the stimulation of mycorrhizal growth and arbuscular phase of the colonization. Studies have revealed that alkaline phosphatase activity is localized in the vacuoles of mature arbuscules (Gianiniazzi et al. 1979). These enzymes (APA and ALA) help in mineralization of bound phosphorus into a soluble form, which makes it more easily available to plants. The present findings are in agreement with numerous other reports (Dodd et al. 1987; Rubio et al. 1990; Abdel-Fattah 2001). Thus, phosphatase activity may be helpful in improving soil health and also act as an indicator of soil quality.

The progressive increase in stomatal conductance resulting from AM inoculation might be due to higher rates of photosynthesis and increase in the number of stomata on the lower surfaces of the leaves. Higher rates of stomatal conductance in mycorrhizal plants are also reported by Auge (2001). Fidelbus et al. (2001) also report that AM fungi have positive role in lowering the rate of leaf conductivity of citrus seedlings growing in very dry soils.

The greater number of nodules number of treated plants may be due to the mutual positive action of *Rhizobium* species and AM fungi strains in increasing the uptake of phosphorus and nitrogen from soil. The role of arbuscular mycorrhizae in improving nodulation and N_2 fixation is universally recognized. AM fungi are known to release an unidentified diffusional factor (myc factor) (Parniske 2008; Maillet et al. 2011), which activates the nodulation factor's inducible gene, involved in establishing the symbiosis with the nitrogen fixing rhizobial bacteria (Kosuta et al. 2003). It is also reported that effective mycorrhizal colonization can also increase nodulation and symbiotic nitrogen fixation in legumes (Hamel 2004). Moreover, when *Rhizobium* bacteria are present in the soil, mycorrhizal colonization is increased due to an increase in the concentration of chemical signals involved in the establishment of symbiosis (Hirsch and Kapulnik 1998).

The AM prompted nutrient uptake may be due to an increase in surface area of soil contact, increased movement of nutrients into mycorrhizae, a modification of the root environment and increased storage. The effect of the mycorrhizal infection of the host plant is an increase in the uptake of P, which is mainly due to the capacity of the mycorrhizal fungi to absorb phosphate from soil and transfer it to the host roots. Khan et al. (2008) also found an increase in both N and P followed by higher yield in *Medicago sativa* inoculated with *Gigaspora rosea* and *Glomus intraradices*. In addition, Safapour et al. (2011), report similar increases in yield of three varieties of red bean when inoculated with AM fungi and *Rhizobium* sp.

Conclusions

In conclusion, dominant arbuscular mycorrhizal fungi, either alone or in combination, can establish a symbiosis with mung bean, which results in this plant growing better and producing more beans under laboratory conditions. There is now a need to determine whether this technique can be used for increasing the growth and yield of mung bean under field conditions.

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COMPATIBLE AND INCOMPATIBLE POLLINATION AND THE SENESCENCE AND OVARY GROWTH OF *DENDROBIUM* FLOWERS

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ABSTRACT

The pollinia from five cultivars of *Dendrobium* ('Miss Teen', 'Karen', 'Sakura', 'Willie', and 'Pompadour') were placed on the stigma of open flowers of *Dendrobium* 'Miss Teen' (or 'Kenny'). The ethylene production and post-pollination development of cross-pollinated flowers were monitored. The pollinated flowers showed two different development patterns depending upon pollination compatibility. The first group, which was pollinated with incompatible pollen from flowers of 'Karen' and 'Miss Teen', did not exhibit post-pollination symptoms and ovary growth. The second group, which was pollinated with compatible pollen from flowers of 'Pompadour', 'Sakura' and 'Willie', showed premature perianth senescence and induced ovary growth within a day. Compatible pollination of 'Miss Teen' flowers resulted in an immediate burst in the production of ethylene and a rapid ovary growth followed by an increase in pollen tube length. In contrast, when 'Miss Teen' flowers were self-pollinated or pollinated with 'Karen' pollinia, these changes did not occur. In the pollinia of 'Pompadour', 'Sakura' and 'Willie' there were higher levels of auxin and 1-aminocyclopropane-1-carboxylic acicd (ACC) than in those of 'Miss Teen' and 'Karen'. Compatible pollination also resulted in higher ACC synthase and ACC oxidase activities within 12 h after pollination than after pollination with incompatible pollen. There was a low level of ACC oxidase activity in non-pollinated flowers and a higher ACC oxidase activity in the column plus pedicel than petal plus sepal and lip.

Keywords: ethylene production, ovary growth, auxin, 1-aminocyclopropane-1-carboxylic acid, senescence

Introduction

Pollination is important for the successful sexual reproduction of flowering plants. Compatibility of the pollen is crucial for the production of seeds of newly inbred orchids or rare species in commercial propagation (Aggarwal et al. 2012; Cevdet and Sebnem 2012; Damon et al. 2012) In addition to ovary growth and seed development, pollination induces early senescence of the flowers of many species of carnation, cyclamen, petunia, tobacco, and orchids (Herrero and Dickinson 1980; Halevy et al. 1984; Hill et al. 1987; Stead 1992; Halevy 1995a; Porat et al. 1995; Porat et al. 1998; Ketsa and Rugkong 2000; Xu and Hanson 2000; Ketsa et al. 2001). Generally, pollination can be divided into three stages: 1) contact of pollen with the stigma, 2) growth of pollen tubes into the style and 3) fertilization of ovules (Halevy 1995b).

The early senescence of the perianth following pollination is accompanied by an increase in the production of ethylene (Woodson and Lawton 1988; Porat et al. 1995; Woltering et al. 1995). The gaseous hormone ethylene is produced by all parts of higher plants, with the rate of production dependent on tissue type and the stage of development. Ethylene biosynthesis is most active in meristematic and nodal regions and also increases during leaf abscission, flower senescence and the ripening of fruit (Have and Woltering 1997). Auxin, wounding and physiological stress, associated with flooding, chilling, disease, high temperature or drought, can induce the biosynthesis of ethylene (Woodson et al. 1992). The biosynthetic pathway resulting in the production of ethylene by pollinated flowers is the same as in ripening fruit and wounded tissue. In the first step, methionine is converted to SAM (S-adenosyl methionine) by SAM synthase, which is then converted to ACC (1-aminocyclopropane-1-carboxylic acid) by ACC synthase, which is oxidized to ethylene by ACC oxidase (Yang and Hoffman 1984; Taiz and Zeiger 1998).

Self-incompatibility (SI) is a reproductive strategy of flowering plants that enables the pistil to reject genetically related pollen. In contrast, pollen from other plants is accepted by the pistil and the pollen tube grows down through the style to reach the ovary where fertilization occurs (McCubbin and Kao 2000). SI is found in many species of plants, such as petunia (Herrero and Dickinson 1979) where compatible pollination leads to rapid senescence. Petunia flowers pollinated with incompatible pollen last almost as long as non-pollinated flowers. After pollination compatible pollen contains a higher content of ACC and induces higher levels of ethylene production than incompatible pollen (Singh et al. 1992). Baker et al. (1997) also report that in *Theobroma cacao* L., IAA concentrations increase in compatibly pollinated flowers but remain stable in incompatibly pollinated flowers. Although SI occurs in *Dendrobium* the physiological changes following pollination with compatible and non-compatible pollen, however, has not been reported.

Dendrobium is a very large genus of orchids established by Olof Swartz in 1799 and today contains about 1,200 species. The genus occurs in diverse habitats throughout much of south, east and southeast Asia, including the Philippines, Borneo, Australia, New Guinea, Solomon Islands and New Zealand. *Dendrobium* orchids are the most important cut-flowers exported by Thailand. To meet commercial needs, new cultivars of *Dendrobium* orchids are developed by amateur and scientific breeders. However, SI is frequently encountered when attempting self- and cross-pollination. In a preliminary study we have shown that cut flowers of *Dendrobium* kept in test tubes containing sugar and an antimicrobial compound can be pollinated successfully and develop pods with seeds that germinate to produce plantlets. This method is suitable for use in breeding programs as it enables one to cross many plants in conditions where it is possible to control the temperature, relative humidity and light.

Here, our results of controlled pollination experiments with cut flowers of *Dendrobium* are reported. Pollination by compatible pollen is followed by early perianth senescence and ovary growth whereas that by incompatible pollen by delayed senescence and no ovary growth. The objectives of this study were: (i) to compare the physiological changes and ethylene production following compatible and incompatible pollination of cut flowers of *Dendrobium*, and (ii) to determine the ethylene production by pollinated flowers of *Dendrobium* following pollination by compatible and incompatible pollen.

Materials and methods

Inflorescences of *Dendrobium* 'Miss Teen' or 'Kenny', *Dendrobium* 'Sakura', *Dendrobium* 'Pompadour', *Dendrobium* 'Willie' and *Dendrobium* 'Karen' were purchased from a suburban Bangkok grower. The inflorescences with five to six open flowers and three to five flower buds were harvested in the morning and returned to the laboratory within 2 h of harvest. The peduncle and attached buds were cut off and only the first five open flowers were used. The peduncle of each inflorescence was cut at an angle, 12 cm from the base of the first open flower and the cut end inserted into a 15-ml centrifuge tube containing 10 ml of distilled water. These inflorescences were kept under natural light (about 15 μmol m^{-1} s^{-1}), ambient temperature (average 25 °C) and relative humidity (average 70% RH) conditions during the period of the study.

Identification of compatible and incompatible pollinia

Open flowers of *Dendrobium* 'Miss Teen' were used as the female parent. The open flowers were hand pollinated by placing the pollinia from 5 cultivars of *Dendrobium*, namely 'Miss Teen', 'Karen, 'Pompadour', 'Sakura' and 'Willie', onto the stigma without removing the anther cap and pollinia. There were 25 replicates of each experiment (one flower/replication) and six treatments as follows:

1. Non-pollinated flowers of *Dendrobium* 'Miss Teen' as the control
2. *Dendrobium* 'Miss Teen' × *Dendrobium* 'Miss Teen'
3. *Dendrobium* 'Miss Teen' × *Dendrobium* 'Karen'
4. *Dendrobium* 'Miss Teen' × *Dendrobium* 'Pompadour'
5. *Dendrobium* 'Miss Teen' × *Dendrobium* 'Sakura'
6. *Dendrobium* 'Miss Teen' × *Dendrobium* 'Willie'

In treatments 2 and 3, pollination was incompatible, whereas in treatments 4, 5 and 6 it was compatible. Post-pollination developmental events were observed and recorded as follows:

1. Symptoms of senescence were noted. The changes in the pollinated flowers and the time to onset of senescence indicated by epinasty (the pollinated flowers turn upside down), drooping, venation, fading, lip yellowing and petal senescence of individual open flowers were recorded daily.
2. Distance between lip and peduncle in cm (Ketsa and Rugkong 1999).
3. The ovary diameter (mm) was determined at the proximal end, using a caliper.
4. Ethylene production.

To measure ethylene production, the cut ends of two inflorescences bearing only 4 open flowers from the bottom were weighed and the cut ends inserted into centrifuge tubes containing 10 ml of distilled water and placed into an empty, air-tight 4 l bottle with gas sampling ports. At various intervals the bottles were sealed for 2 h and a 1 ml gas sample was withdrawn from the headspace for ethylene determination using a gas chromatograph equipped with a flame ionization detector (Shimadzu GC-14A). After each determination, all the inflorescences were removed from the bottles.

Indoleacetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC) content of pollinia

To analyze IAA and ACC content, incompatible and compatible pollinia were collected from open flowers for extraction and analysis. Pollinia of 'Karen' were incompatible, while those of 'Sakura' were compatible. There were three replicates for each cultivar.

ACC was extracted and analyzed following the method of Lizada and Yang (1979) modified by Hoffman and Yang (1982). Pollinia were weighed (0.03 g) and ground in 5 ml of 9% trichloroacetic acid (TCA) using a mortar and pestle. ACC content was measured in terms of the oxidative release of ethylene, as described by Lizada and Yang (1979) Ethylene levels were measured in 1.0 ml of the headspace after 5 min. To measure the efficiency of conversion of ACC to ethylene in the extracts, 0.5 ml of the extract was spiked with 0.4 nmol of ACC to

Table 1 Time to petal senescence of non-pollinated flowers of *Dendrobium* 'Miss Teen' and those pollinated with pollinia from five other cultivars.

Treatment	Epinasty	Droop	Venation	Lip yellowing	Fading	Browning
'Miss Teen' (No pollination)	>15.6[a]	>16.2[a]	>17.0[a]	>17.0[a]	>17.0[a]	>17.0[a]
'Miss Teen' × 'Miss Teen'	>12.8[b]	>14.7[b]	>15.9[b]	>16.1[ab]	>16.5[a]	>16.5[a]
'Miss Teen' × 'Karen'	>9.8[c]	>11.2[c]	>16.1[b]	>16.4[b]	>17.0[a]	>17.0[a]
'Miss Teen' × 'Pompadour'	1.1[d]	3.1[d]	4.4[d]	6.1[d]	8.2[b]	9.3[b]
'Miss Teen' × 'Willie'	1.1[d]	3.7[d]	6.3[c]	7.1[c]	8.0[b]	8.0[d]
'Miss Teen' × 'Sakura'	1.1[d]	2.7[d]	4.0[d]	4.2[d]	6.0[c]	8.6[c]
F-test	**	**	**	**	**	**

Means within columns not sharing the same letter are significantly different at $P = 0.01$ (DMRT) and ** = significant at $P = 0.01$

give a volume 0.9 ml after the addition of the $HgCl_2$. The ethylene production was measured after the addition of the bleach base solution as described above. The conversion efficiency of freshly made floral extracts was found to be close to 100%. ACC content was expressed as nmol ACC formed per g FW.

IAA in pollinia

IAA in pollinia was extracted and analyzed following the method of Abdel-Rahman et al. (1975). Three hundred mg of pollinia were extracted in 15 ml cold methanol (95%). The residue after extraction and separation of the ethyl acetate was evaporated and the residue dissolved in 1 ml of 100% methanol and the IAA in the purified extract was determined using high pressure liquid chromatography (HPLC).

High-performance liquid chromatographic analysis of indole-acetic acid

One ml of pollen extract was filtered through a Nylon Acrodisc 13 (0.45 µm). The HPLC analyses were carried out using a Shimadzu class LC-VP HPLC system with LC-VP software, a pump (LC-10AD VP) and a UV detector (SPD-10A VP) (Shimadzu, Kyoto, Japan). The separation was carried out on a Alltima C18 column 5 µ (250 × 4.6 mm) Lot No. 2672 (Alltech, USA) with guard column (CLC-ODS(4), 10 × 4 mm I.D.), using 30% methanol and 0.8% acetic acid as the mobile phase at a flow rate 1.5 ml/min and monitored at 280 nm. The retention time for IAA peak was 12.8 min.

Results

Time of senescence following pollination with different pollinia

Dendrobium 'Miss Teen' flowers were either self-pollinated or pollinated with pollinia from four other cultivars and non-pollinated flowers served as the control. Two types of behaviour were observed. Early symptoms of senescence (epinasty, lip yellowing, venation and water soaking) and early ovary growth were recorded

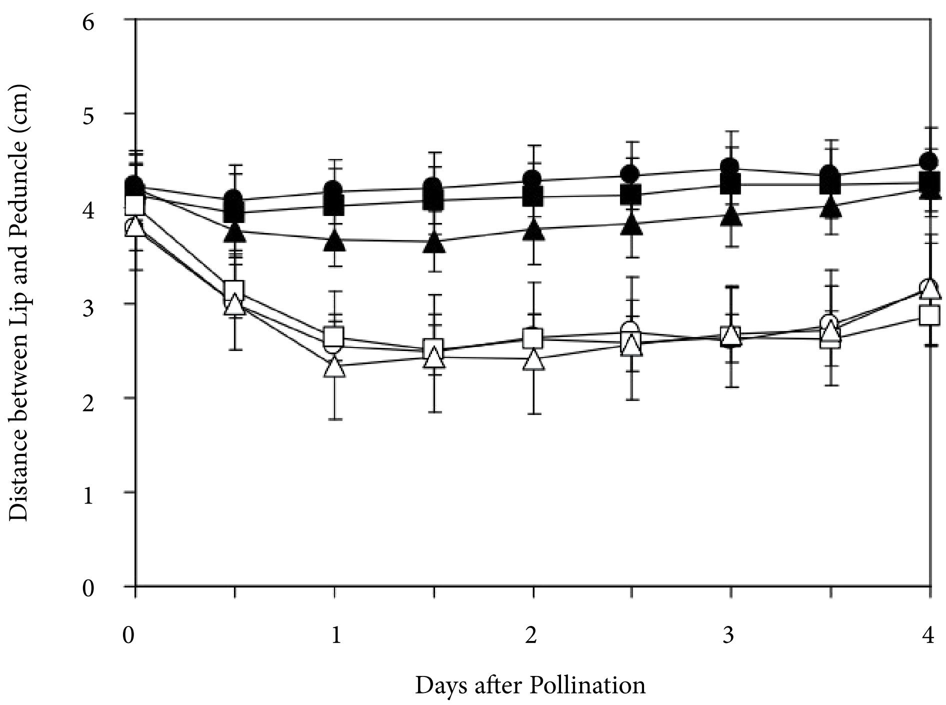

Fig. 1 Changes in distance between lip and peduncle of non-pollinated *Dendrobium* Miss Teen' flowers (●) and those pollinated with pollinia from 'Miss Teen' (■), 'Karen' (▲), 'Pompadour' (○), 'Sakura (□) and 'Willie' (Δ). Results are means of 25 flowers ± SD.

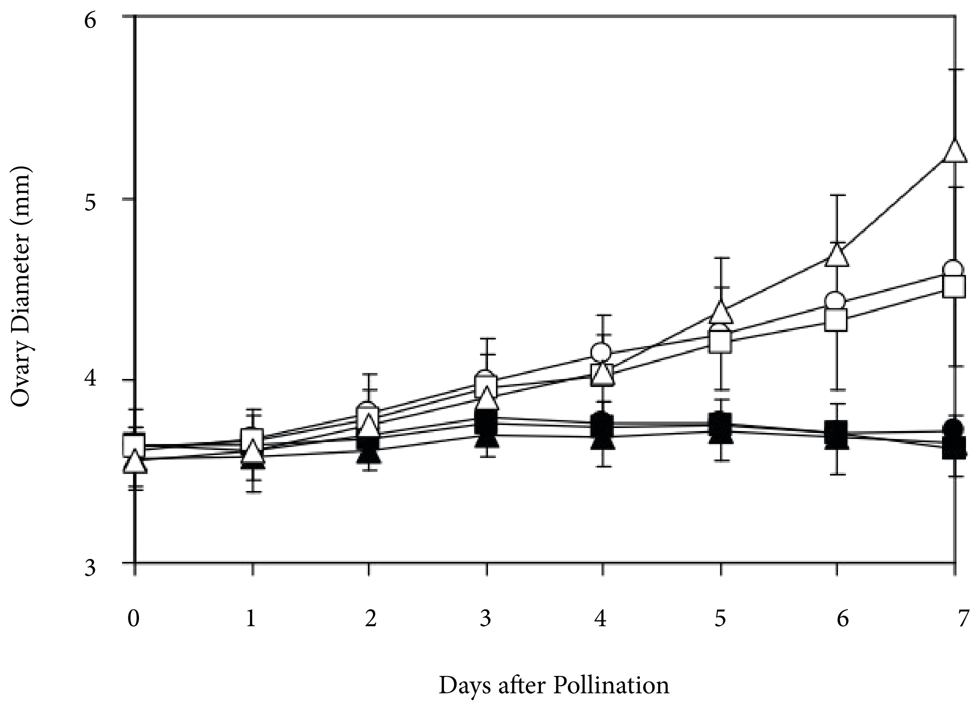

Fig. 2 Ovary growth of non-pollinated *Dendrobium* 'Miss Teen' flowers (●) and those pollinated with pollinia from 'Miss Teen' (■), 'Karen' (▲), 'Pompadour' (□), 'Sakura (○) and 'Willie' (Δ). Results are means of 25 flowers ± SD.

when *Dendrobium* 'Miss Teen' flowers were cross-pollinated with compatible pollinia from 'Pompadour', 'Sakura' and 'Willie' cultivars (Table 1). The second type of behaviour in which there was delayed senescence and ovary growth was recorded after self-pollination of 'Miss Teen' flowers or after cross-pollination of 'Miss Teen' flowers with incompatible 'Karen' pollinia. The delay in senescence was similar to that recorded for non-pollinated flowers. The senescence of flowers was expressed in terms of several symptoms that developed sequentially. Flower epinasty was the earliest of the senescence symptoms and occurred within 1-2 days in the case of compatible pollination. Drooping developed 2–3 days after pollination (DAP) followed by colour fading, yellowing of lip and water soaking (data not shown). Lip yellowing, fading and browning following incompatible pollination were the same as that recorded for non-pollinated control flowers but epinasty, droop-

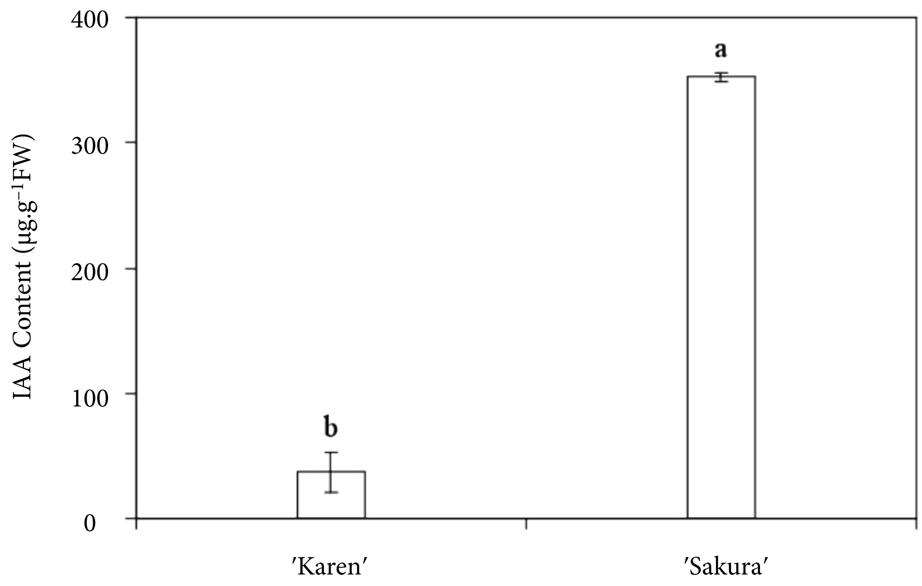

Fig. 3 Content of IAA in pollinia from *Dendrobium* 'Karen' and 'Sakura'. Results are means of 3 replicates ± SD. Different letters indicate significant differences at $p \leq 0.05$ based on *t*-tests.

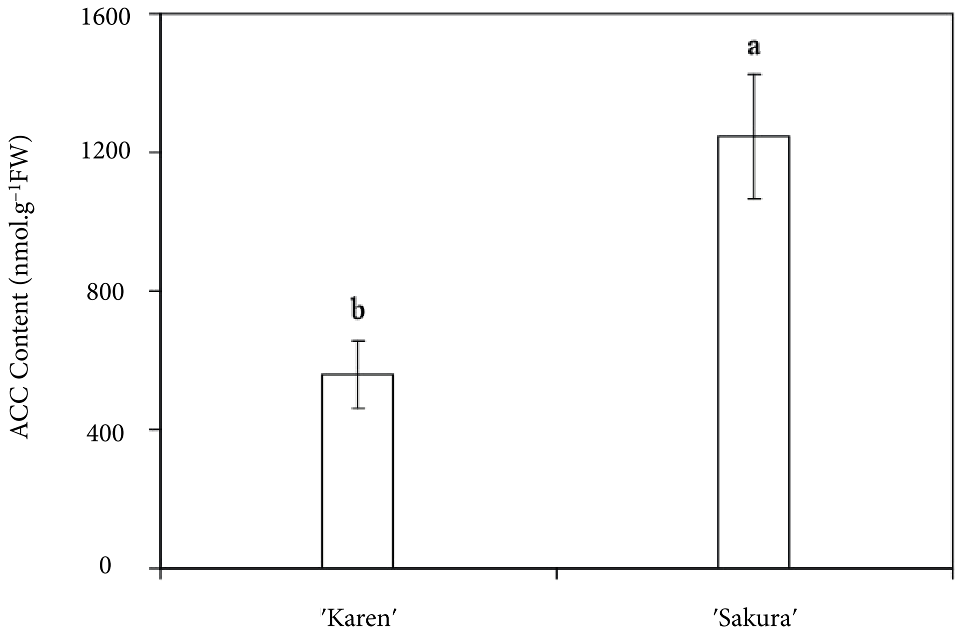

Fig. 4 Content of ACC in pollinia from *Dendrobium* 'Karen' and 'Sakura'. Results are means of 3 replicates ± SD. Different letters indicate significant differences at $p \leq 0.05$ based on *t*-tests.

ing and changes in the venation occurred slightly faster (Table 1).

When pollinia of 'Pompadour', 'Sakura', and 'Willie' were placed on the stigma of 'Miss Teen' the column (tissue around the stigma) was observed to rapidly swell within 1 DAP. The colour of the pedicel changed from white to green accompanied by the enlargement of the proximal end of the pedicel. These changes were not recorded for non-pollinated flowers, self-pollinated 'Miss Teen' flowers or those pollinated with pollinia from 'Karen'.

Effects of pollination on the distance between lip and peduncle

Within 12 hours of pollination (HAP) the distance between lip and peduncle changed. The distance between the lip and peduncle of flowers of 'Miss Teen' pollinat-

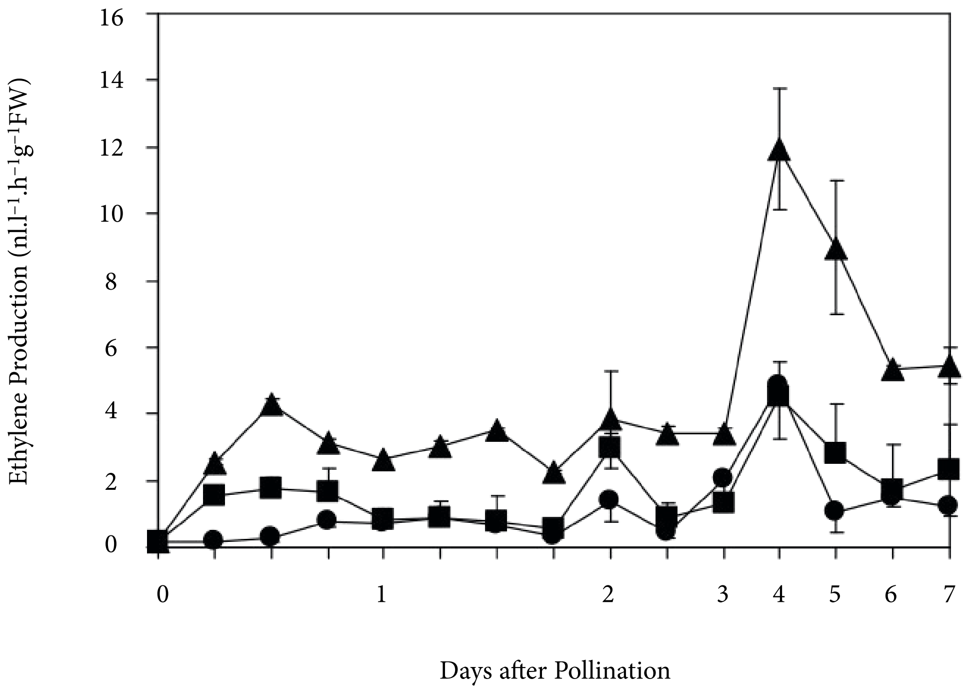

Fig. 5 Ethylene production by non-pollinated flowers of *Dendrobium* 'Miss Teen' (●) and those compatibly pollinated (× 'Sakura') (▲) and incompatibly pollinated (× 'Karen') (■). Results are means of 10 replicates ± SD.

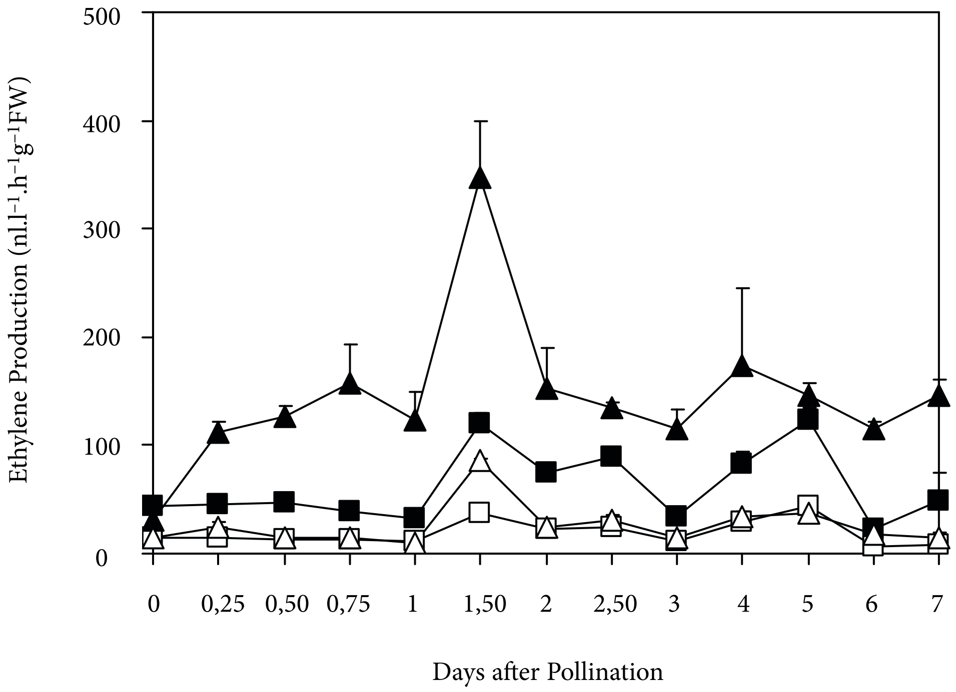

Fig. 6 Ethylene production by the column plus pedicel of non-pollinated (■), petal, sepal plus lip of non-pollinated (□),column plus pedicel of compatibly pollinated (▲) and petal, sepal plus lip of compatibly pollinated (Δ) flowers of *Dendrobium* 'Miss Teen'. Results are means of 5 replicates ± S.

ed with compatible pollinia ('Pompadour', 'Sakura' and 'Willie') rapidly decreased, which resulted in the epinasty of the flowers (Fig. 1). The distance between lip and peduncle in the case of incompatible pollination was only slightly less but epinasty of the flowers did not occur. The distance between lip and peduncle of non-pollinated flower remained unchanged (Fig. 2).

Ovary diameter

The diameter of the ovary of flowers of 'Miss Teen' either non-pollinated or self-pollinated or pollinated with pollinia from 'Karen' remained unchanged over the experimental period. In contrast, compatible pollination resulted in a steady increase in the diameter of the ovary over the 7-day period of the experiment (Fig. 2).

Quantity of IAA and ACC in pollinia

The incompatible pollinia of 'Karen' contained low amounts of IAA and ACC, while the compatible pollinia of 'Sakura' contained relatively high amounts of both IAA and ACC (Figs. 3 and 4, respectively).

Ethylene production

Compatibly pollinated flowers (× 'Sakura') produced more ethylene than incompatibly pollinated flowers (× 'Karen'). The ethylene production of compatibly pollinated flowers increased and peaked within 12 HAP and remained high and peaked again on day 4, whereas non-pollinated flowers produced very little ethylene (Fig. 5). The high levels of ethylene induced by pollination were produced by the column plus pedicel and the petal, sepal plus lip produced very little ethylene (Fig. 6).

Discussion

Pollination with pollinia from *Dendrobium* 'Sakura' stimulated a high level of ethylene production and subsequently the early senescence of the perianth of the flowers of *Dendrobium*. The results using 'Miss Teen' are similar to those for *Dendrobium* 'Pompadour' (Ketsa and Rugkong 2000), *Phalaenopsis* (Zhang and O'Neill 1993), petunia (Singh et al. 1992) and tobacco (Hill et al. 1987). Following pollination by pollinia from cultivar 'Sakura' the ovary grew considerably but there was little or no effect after pollination with pollinia from *Dendrobium* 'Karen'. In this study, ethylene production following compatible and incompatible pollination was clearly different. Ethylene production and ovary growth increased after pollination with compatible pollinia.

Pollinia of *Dendrobium* are known to be rich in auxin (IAA) and ACC (Arditti 1979; Stead 1992). However, the concentrations of IAA and ACC in 'Karen' pollinia was low and in 'Sakura' pollinia relatively high. The variation in the IAA and ACC contents of pollinia, however, depends on the genetic background, may also account for the difference in the compatible and incompatible pollinia. Pollinia of 'Sakura', which were compatible, contained high levels of IAA and ACC, while those of 'Karen', which were incompatible, contained low amounts of IAA and ACC. Baker et al. (1997) report that in *Theobroma cacao* L., IAA concentrations in flowers pollinated with compatible pollinia increase but remain stable in those pollinated with incompatible pollinia.

The bursts of ethylene production, ovary growth and premature senescence were triggered by cross-pollination with pollinia from 'Sakura' but not by cross-pollination with incompatible pollinia from 'Karen'. The major factor determining why cross pollination with compatible pollinia induces high levels of ACC and IAA in the pollinia, which seems to stimulate ovary development, is apparently related to pollen germination (Singh et al. 1992; Luangsuwalai et al. 2008). It is unknown whether premature senescence is also directly induced by these two chemicals, which may act directly on the petals or via their effect on the ovary. A second part of the causal chain may be the differences in pollen germination and pollen tube growth. Both seem to require ethylene because application of AOA and/ or 1-MCP to the stigma before compatible cross-pollination reduces pollinia tube and ovary growth (Luangsuwalai et al. 2008). It is also unknown to what extent they are required for the induction of premature senescence. In contrast, in *Phalaenopsis* the pollination-induced senescence syndrome is not associated with pollen germination as the pollen starts to germinate 7 days after pollination, which is long after the petals have completely wilted (Zhang and O'Neill 1993). In tomato, ethylene promotes pollen germination and pollen tube growth (McLeod 1975) and increases germination and pollen growth in pear (Search and Stanley 1970) and peach (Buchanan and Biggs 1969). De Martinis et al. (2002) have demonstrated that the production of ethylene by flowers of tobacco upon pollination is a direct response to pollen tube growth.

Using the compatible pollinia from *Dendrobium* 'Sakura' and the incompatible pollinia from *Dendrobium* 'Karen' for pollination revealed that flowers pollinated with compatible pollinia produced a burst of ethylene. Pollination with compatible pollinia not only induce an increase in ethylene production but also an increase in sensitivity to ethylene (Porat et al. 1994). Flowers pollinated with incompatible pollinia produced low levels of ethylene. The pattern in the ethylene production by flowers pollinated with compatible and incompatible pollinia, and non-pollinated flowers were similar to that reported for petunia (Singh et al. 1992). Incompatible pollination did not stimulate early senescence as fast as compatible pollination (Table 1). In compatible pollination, it is possible that the stigma perceived the primary pollination signal from pollinia and then transmitted and amplified this primary signal, which coordinates inter-organ post-pollination responses (O'Neill 1997). Incompatible pollination slightly affected ethylene production, which then decreased and remained low, similar to that recorded in non-pollinated flowers. It seems that the signal from incompatible pollination was not strong enough to elicit early petal senescence, such as lip yellowing and fading. The results reported here raise several interesting questions about the role of pollination-induced ethylene. Ketsa et al. (2001) report that *Dendrobium* flowers pollinated with pollen from plants other than *Dendrobium*, produced levels of ethylene similar to flowers pollinated by compatible pollen, but no growth in the ovary. Therefore, pollination-induced ethylene production alone does not promote pollen tube and ovary growth, which may be due to other pollination-induced factors (Singh et al. 1992).

It is concluded that *Dendrobium* flowers can be pollinated by both compatible and incompatible pollinia. Pollination by compatible pollinia induces early perianth senescence and ovary growth but pollination by incompatible pollinia does not result in early senescence of the perianth or ovary growth. Compatible pollinia contain greater amounts of auxin and ACC, and induce a greater production of ethylene than incompatible pollinia.

Acknowledgements

The research was financially supported by the Thailand Research Fund (TRF), the Commission on Higher Education (CHE), Ministry of Education and the Kasetsart University Research and Development Institute (KURDI).

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ROLE OF STIGMATIC FLUID IN OVARY GROWTH AND SENESCENCE OF POLLINATED *DENDROBIUM* FLOWERS

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ABSTRACT

Pollination induced an increase in ethylene production concomitantly with ovary growth, epinasty, drooping, changes in venation and fading of the colour of the flowers of *Dendrobium* 'Pompadour'. Removal of stigmatic fluid prior to pollination significantly reduced ovary growth, while ethylene production, epinasty, drooping, venation and colour of pollinated flowers were not significantly affected. Removal of stigma fluid had no effect on ovary growth of non-pollinated flowers treated with 1-aminocyclopropane-1-carboxylic acid (ACC), but significantly reduced the ovary growth of non-pollinated flowers induced by the application of napthaleneacetic acid (NAA). The effect of stigmatic fluid on ovary growth of pollinated flowers is discussed.

Keywords: stigmatic fluid, ethylene production, ovary growth, senescence

Introduction

Many flowers show early petal senescence after pollination (O'Neill 1997; O'Neill and Nadeau 1997). In the case of the flowers of the orchid *Dendrobium*, non-pollinated flowers senesce after up to 3–4 weeks, whereas pollination is rapidly followed by visible symptoms of senescence (Ketsa and Rugkong 1999). The direct cause of *Dendrobium* flower senescence following pollination is the pollination-induced increase in ethylene production (Ketsa and Luangsuwalai 1996; Ketsa and Rugkong 2000a; Ketsa et al. 2001). Pollination induces a syndrome of developmental events that includes epinasty of the flower stalk, flower pigmentation and growth of the ovary (Ketsa and Rugkong 2000a; Ketsa and Rugkong 2000b; Ketsa et al. 2001).

The stigma in many species exudes an aqueous substance. This stigmatic fluid consists of water, sugars, fatty acids and amino acids (Konar and Linskens 1966; Martin 1973; Lovell et al. 1987; Lack and Diaz 1991). This fluid seems to aid the adherence of pollen to the stigma and to promote both pollen germination and pollen tube growth. Konar and Linskens (1966) also report that this fluid is important for ovary growth.

The present study investigates the role of stigmatic fluid in post pollination development, including ovary growth, of *Dendrobium* flowers. It is suggested that the increase in ethylene production and several other early post pollination changes in orchids, are due to the presence of ACC (the direct precursor of ethylene) and auxin-like compounds in pollinia (O'Neill 1997; Porat et al. 1998). In this study we investigated the effects of applying ACC and an auxin (NAA) to the stigma of non-pollinated flowers from which the stigma fluid was removed.

Materials and methods

Inflorescences of *Dendrobium* 'Pompadour' were purchased from a commercial grower near Bangkok. They consisted of five to seven open florets and five or six flower buds. Inflorescences were harvested in the morning and brought to the laboratory within 2 h of harvest. The upper part of the stem of the inflorescence with attached buds was removed and only the first five open florets were used. The stem at the base of the inflorescence was cut at an angle, 12 cm from the first open floret and the cut end inserted into a 15-ml centrifuge tube containing 10 ml of distilled water. Inflorescences were kept at ambient temperature (29.5 ± 2 °C) and relative humidity (76 ± 3% RH), and under natural light conditions.

Flowers were cross-pollinated by placing the pollinia from other open florets of *Dendrobium* 'Pompadour'. Prior to pollination or treatment with chemicals the stigmatic fluid was gently removed from open florets, using a small metal scoop specially made for this experiment.

1-Aminocyclopropane-1-cyclopropane acid (ACC) (20 nmol per floret) or napthaleneacetic acid (NAA) (20 µg per floret) was applied in distilled water to the stigma surface of non-pollinated flowers, with and without stigma fluid. The same volume of distilled water was applied to the stigma surface as a control.

The ovary diameter was determined at the proximal end, using a calliper. Orchid flowers exhibited peduncle epinasty after pollination, which was measured in terms of the distance between the lip and pedicel (Ketsa and Rugkong 1999). The time to epinasty, drooping, venation and colour fading of individual open florets was recorded daily.

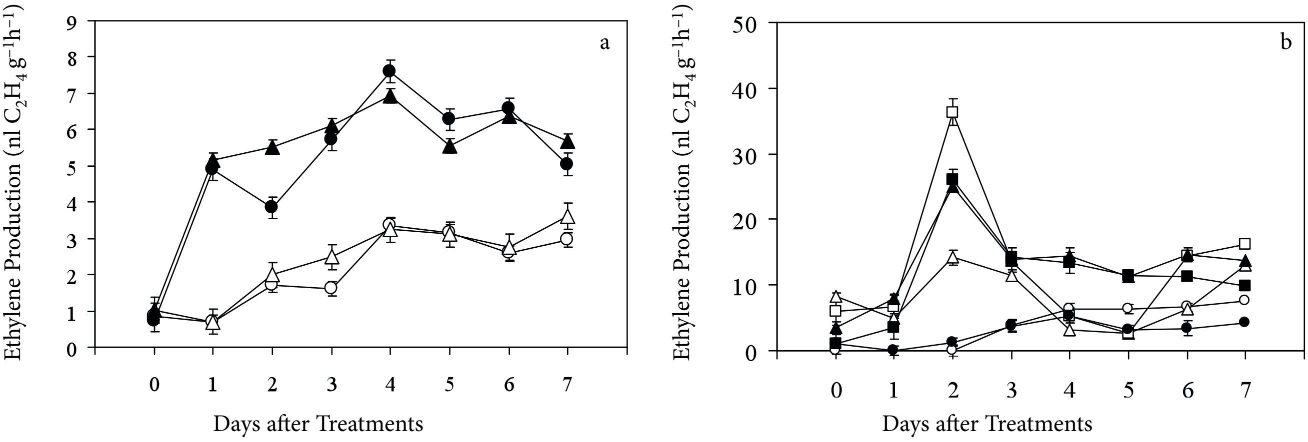

Fig. 1 Production of ethylene by flowers: (a) ethylene production of non-pollinated (○, Δ) and pollinated (●, ▲) flowers with (○, ●) and without (Δ, ▲) stigmatic fluid; (b) ethylene production of non-pollinated flowers with (○, Δ, □) and without (●, ▲, ■) stigmatic fluid and treated with distilled water (○, ●), NAA (□, ■) or ACC (Δ, ▲). Values are means for 25 open florets ± SE.

Individual inflorescences were weighed and placed in airtight, 4 litres glass bottles with gas sampling ports. At various intervals, bottles were sealed for 2 h and samples of 1 ml gas were withdrawn from the headspace for ethylene determination using a gas chromatograph equipped with a flame ionization detector (Shimadzu, Kyoto, Japan). After each determination, the bottles were opened for aeration. There were 10 replicates of each treatment.

Five inflorescences were used in each treatment and each inflorescence bore five open florets. Where possible, means were compared using Duncan's new multiple range test (DMRT). The experiments were repeated at a later date.

Results

Ethylene production by non-pollinated flowers with and without stigmatic fluid steadily increased during the period of the study. Ethylene production of pollinated flowers increased much more rapidly (Fig. 1a). Removal of stigmatic fluid had no significant effect on ethylene production of flowers, either non-pollinated or pollinated (data not shown).

Application of NAA or ACC to the stigma resulted in an increase in the production of ethylene, which peaked on day 2 (Fig. 1b). Treatment with NAA resulted in a higher production of ethylene than that with ACC. On day 2 the production of ethylene by NAA-treated flowers with stigmatic fluid was greater than that of NAA-treated flowers without stigmatic fluid. The opposite was recorded when treated with ACC (Fig. 1b).

The ovaries of non-pollinated flowers with and without stigmatic fluid did not grow, whereas those of similarly treated pollinated flowers steadily grew throughout the period of the study. Ovary growth of pollinated flowers with stigmatic fluid increased more rapidly than that of flowers from which the fluid was removed (Fig. 2a). This difference in the rate of growth was statistically significant at the end of the study period, (Table 1).

Ovary growth of NAA-treated non-pollinated flowers with and without stigmatic fluid rapidly increased throughout the study period. This growth was greater in flowers with stigmatic fluid (Fig. 2b; Table 2). The ovaries of ACC-treated flowers with and without stigmatic fluid did not increase in size (Fig. 2b).

Epinasty of the flower pedicel is here shown to result from a decrease in the distance between the lip and peduncle that occurs within 0–2 days of pollination (Fig. 3a). The later change (increase in distance) is due to the inward folding (called drooping) of the petals, in-

Table 1 Size of the ovaries of flowers of *Dendrobium* 'Pompadour' from which, before pollination, the stigmatic fluid was either removed or not removed.

Treatment	Ovary size (mm)[1]	
	Day 0	Day 7
Non-pollinated flowers from which the stigmatic fluid was not removed	2.72	2.71c
Non-pollinated flowers from which the stigmatic fluid was removed	2.79	2.74c
Pollinated flowers from which the stigmatic fluid was not removed	2.86	3.87a
Pollinated flowers from which the stigmatic fluid was removed	2.84	3.49b
F-test	ns	**

[1]Means within a column not sharing common letter are significantly different at $P = 0.01$ based on DMRT; ns = not significant, ** = significant at $P = 0.01$ and n = 25.

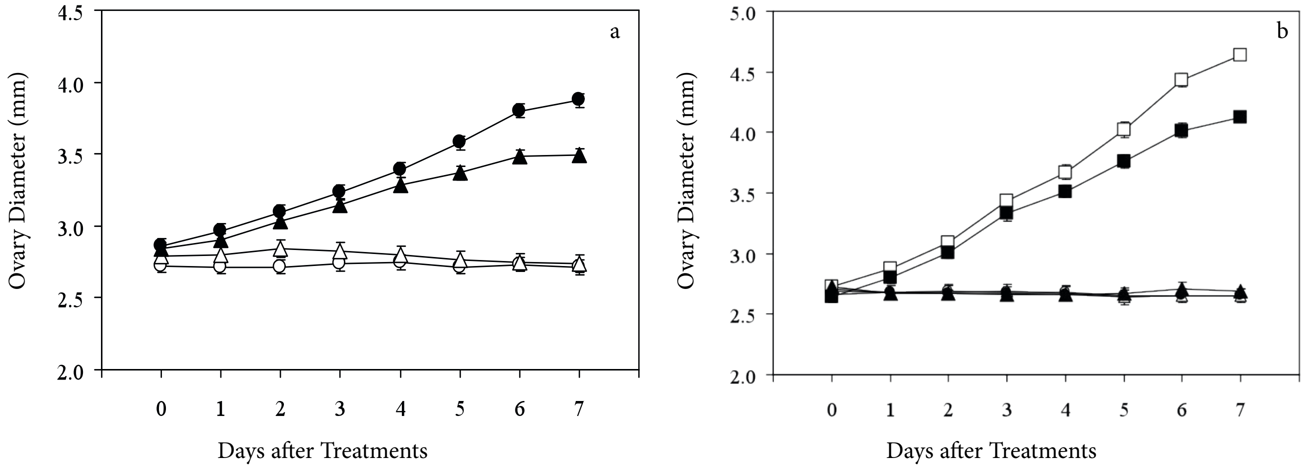

Fig. 2 Ovary growth of: (a) non-pollinated (○, Δ) and pollinated (●, ▲) flowers with (○, ●) and without (Δ, ▲) stigmatic fluid; (b) non-pollinated flowers with (○, Δ, □) and without (●, ▲, □) stigmatic fluid and treated with either distilled water (○, ●), NAA (□, ■) and ACC (Δ, ▲). Values are means for 25 open florets ± SE.

cluding the lip. Epinasty did not occur in non-pollinated flowers, whether the stigmatic fluid was removed or not. Epinasty only occurred in pollinated flowers (Fig. 3a). The effect of removing stigmatic fluid on epinasty was not significant (Table 3).

Treatment of non-pollinated flowers with NAA resulted in epinasty similar to that recorded after pollination. Treatment of non-pollinated flowers with ACC resulted in slight epinasty (Fig. 3b). The presence of stigmatic fluid did not have a consistent effect, but the effect of ACC did seem to be greater when stigmatic fluid was present (Fig. 3b).

The petals of pollinated flowers folded inwards, which is indicated by the change in the distance between the lip and peduncle that occurred 3–7 days after pollination (Fig. 3). Drooping was more marked in the presence of stigmatic fluid (Fig. 3a). Treatment of non-pollinated flowers with NAA also resulted in drooping, but there

Table 2 Size of the ovaries of *Dendrobium* 'Pompadour' flowers from which the stigmatic fluid was either removed or not and the stigma then either not treated or treated with distilled water, NAA or ACC.

Treatment	Ovary size (mm)[1]	
	Day 0	**Day 7**
Distilled water with stigmatic fluid present	2.69	2.66 c
20 nmol ACC with stigmatic fluid removed	2.71	2.65 c
20 ug NAA with stigmatic fluid present	2.72	4.64 a
Distilled water with stigmatic fluid removed	2.66	2.65 c
20 nmol ACC with stigmatic fluid removed	2.72	2.69 c
20 ug NAA with stigmatic fluid removed	2.64	4.13 b
F-test	ns	**

[1]Means within columns not sharing the same letter are significantly different at $P = 0.05$ (DMRT); ns = not significant; ** $P<0.01$.

Table 3 Time to epinasty, drooping, venation and colour fading of *Dendrobium* 'Pompadour' flowers from which the stigmatic fluid was either removed or not prior to pollination.

Treatment	Time (days)[1]			
	Epinasty	**Drooping**	**Venation**	**Colour Fading**
Non-pollinated flowers with stigmatic fluid	9.0 a	12.3 a	12.1 a	13.8 a
Non-pollinated flowers with stigmatic fluid removed	8.1 a	9.9 a	10.5 a	11.5 a
Pollinated flowers with of stigmatic fluid	2 .0 b	3.6 b	2.0 b	4.0 b
Pollinated flowers with stigmatic fluid removed	2.4 b	4.7 b	4.0 b	5.0 b
F-test	**	**	**	**

[1]Means within columns not sharing common letter are significantly different at $P = 0.01$ based on DMRT; ** = $P = 0.01$ and n = 25.

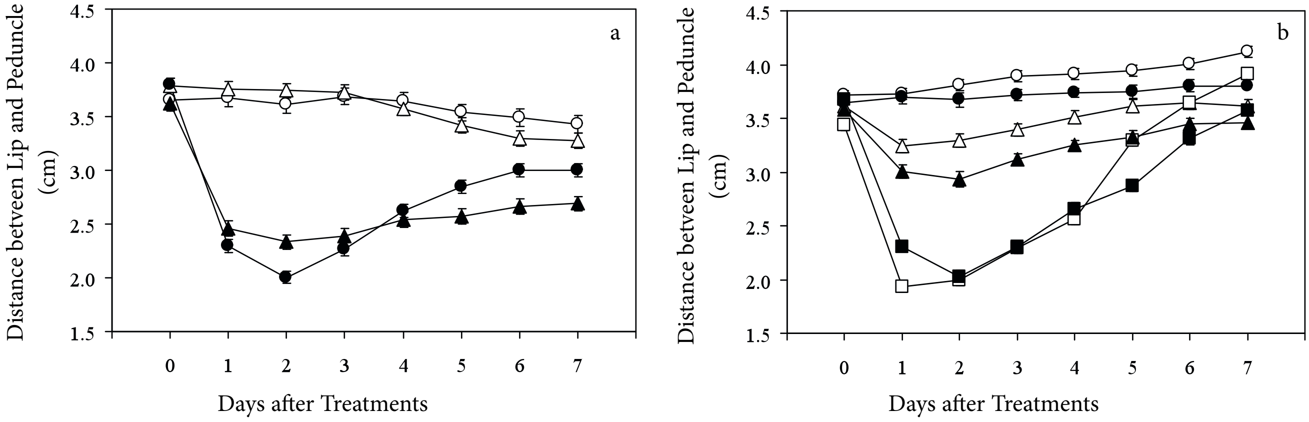

Fig. 3 (a) Distance between the lip and peduncle of non-pollinated (○, Δ) and pollinated (●, ▲) flowers with (○, ●) and without (Δ, ▲) stigmatic fluid. (b) Distance between the lip and peduncle of non-pollinated flowers with (○, Δ, □) and without (●, ▲, ■) stigmatic fluid and treated with either distilled water (○, ●), NAA (□, ■) or ACC (Δ, ▲). Values are means for 25 open florets ± SE. Epinasty of the flower peduncle. Epinasty is shown as the change in distance between the lip and peduncle during day 0–2. This change is due to an inward folding (called drooping) of the petals, including the lip.

was little difference between flowers with and without stigmatic fluid (Fig. 3b).

Symptoms of senescence included a change in petal colour, whereby the colour became less intense (colour fading) and the venation of the petals more visible. Pollination hastened these symptoms (Table 3). The presence or absence of stigmatic fluid had no effect (Table 3).

Discussion

Removal of stigmatic fluid did not significantly affect ethylene production. The absence of an increase in ethylene production indicates that removing stigmatic fluid did not physically damage the surface of the stigma. Removal of stigma fluid prior to pollination resulted in less marked epinasty and a smaller increase in the diameter of the ovary, but these effects were rather small.

In non-pollinated flowers treated with NAA or ACC, removal of stigmatic fluid did not affect the time to the increase ethylene production, but had a significant effect on the magnitude of the ethylene production, with NAA increasing and ACC decreasing ethylene production. It is unknown whether this has a physiological basis. It is suggested that the increase in ethylene production after pollination is due to the presence of ACC and auxin-like compounds. The present data indicate that if this is true the signals released from pollen do not need stigmatic fluid in order to induce an increase in ethylene production. Pollination-induced production of ethylene plays an important role in pollination-induced ovary growth in *Dendrobium* flowers, although an auxin is also required (Ketsa and Rugkong 2000a; Ketsa et al. 2001). Ovary growth of pollinated *Dendrobium* flowers was slightly but significantly less after pollination, if stigmatic fluid was removed. After placing pollinia on the surface of stigma with stigmatic fluid, the pollinia became swollen and firmly embedded in the stigmatic fluid. Substances from the pollen might have dissolved the stigmatic fluid. This could also lead to pollen-pistil interactions resulting in the signals required for ovary growth (Rosen 1975). Another possibility is that the stigmatic fluid stimulated pollen tube growth and therefore ovary growth (Martin 1973).

Acknowledgements

The research was financially supported by the Thailand Research Fund (TRF), the Commission on Higher Education (CHE), Ministry of Education and the Kasesart University Research and Development Institute (KURDI).

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SEASONAL OCCURRENCE AND ABUNDANCE OF WATERBIRDS IN SANDPITS AND FISHPONDS

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ABSTRACT

Censuses of waterfowl at four fishponds and sandpits in the surroundings of Třeboň were carried out regularly during one season and their occurrence was compared with that recorded by other studies and information in the database of the Třeboňsko Protected Landscape Area. Some waterfowl show a marked preference for fishponds during the breeding season. This can be due to many factors, presence of more food, extent of the littoral growth of vegetation, fish stock, depth of water etc. However, sandpits are attractive to waterbirds in winter, when they serve as over wintering sites, meeting places for migrating birds or refuges from hunters.

Keywords: waterbirds, fishponds, sandpits, comparison, occurrence

Introduction

Třeboň Basin is an area, which was reconfigured by humans several hundred years ago by converting most of the natural wetland there into fishponds that are up to several hundred hectares in area and interconnected with each other by a system of drainage streams. The whole region is a Protected Landscape Area (PLA), Biosphere Reserve (BR) and Important Bird Area (IBA), primarily because of the occurrence and nesting there of many species of water and wetland birds.

Currently there are nearly 500 fishponds in the Třeboň Basin covering a total area of over 7200 hectares. The primary function of fishponds is fish farming (mostly common carp – *Cyprinus carpio*) but by their gradual integration into the surrounding landscape, they became suitable habitats for many water animals, water plants and wetland species.

Their shallow depth (up to 1 m) and water rich in nutrients resulted in the development of an extensive littoral vegetation. These shallow reservoirs partly overgrown with aquatic vegetation are suitable habitats for some species of waterbirds (Bezzel 1976; Lutz 2011). Due to intensive fish farming, fishpond trophy and increase in fish stock density the littoral vegetation was restricted (Musil 2000). In the late 70s and early 80s of the 20th century the abundance of waterbirds changed, with some species declining in abundance. The reasons were mainly due to hydrological changes, agricultural intensification, changes in the industrial and urban landscape, human activities and natural vegetation (Hudec et al. 2000).

A relatively new and qualitatively different explanation for the high occurrence of waterbirds in this area are sandpits (Matter and Manna 1998). Unlike fishponds, sandpits are at most 50 years old. The water in sandpits is oligo- or mesotrophic. Unlike fishponds, sandpits are as deep as 22 m and littoral vegetation only sporadically colonizes the steep sides of the sand pits. Recently, some of these sandpits have been used as reservoirs for water supply and some for recreational purposes, including fishing. Barragan-Severo et al. (2002) report that these artificial water reservoirs are important habitats for waterbirds, as they are close to migration routes and used as stepping stones.

Each species chooses reservoirs (fishponds, sandpits) based on the conditions that prevail there (trophy, vegetation type etc.). Some waterbirds use sandpits, when other suitable habitats are lacking (Reitan and Sandvik 1996). Siddle and Kirsch (1993) report the nesting of endangered least tern (*Sterna antillarum*) and piping plover (*Charadrius melodus*) in sandpits in Nebraska, USA. Both of these endangered species found alternate nesting sites around lakes created by sand mining after their natural nesting sites in the river alluvium were mostly destroyed. Nowadays, about 60–90% of the current population of these species nest in sandpits. Hanák et al. (1985) study of the avifauna in sandpits in the Třeboň area reveals that there are 25 species of bird in the Vlkov sandpit (of these 13 are waterbirds) and 40 in the Cep sandpit (of these 16 are waterbirds).

Material and methods

Site characteristics

For avifauna monitoring the Naděje fishpond system (NFS) was chosen (see Balounová et al. 1996 for details). It consists of 15 fishponds of from 1.66 to 63.50 ha in extent, which are used for intensive fish production, mostly carp (*Cyprinus carpio*). There is a zone of littoral vegetation around most fishponds consisting of mainly *Phragmites australis*, *Typha angustifolia* and *Typha latifolia*. One of these fishponds is a nature reserve and the management of the fishery there is less intense.

Monitored sandpits were 13 reservoirs of similar area ranging from 9-123 hectares located at: Veselí – 5 reservoirs, 3 km north of the Naděje fishpond system (NFS), where mining has ceased, Cep 20 km south of NFS with 4 reservoirs (two of them still being mined) and Halámky, 35 km south of NFS, with 4 reservoirs (one of them still being used for extracting sand).

Methods of observation

The populations of waterbirds at four sites in the Třeboň area (an area comparable to the observed fishponds and sandpits) were counted at regular fixed intervals throughout the year. The Naděje fishpond system and Veselí sandpits were monitored throughout the year at two-week intervals, Cep sandpits and Halámky sandpits at four-week intervals. Monitoring of waterbirds at localities was done by counting the birds on the water and by searching for nests in comparable areas of littoral vegetation at these particular fishponds and sandpits (Janda and Řepa 1986).

The monitoring of population abundance involved counting all the birds on the water and other species of birds associated with aquatic and wetland environments – birds of the orders Podicipediformes, Anseriformes, Pelecaniformes, Ciconiiformes, Charadriiformes, Gruiformes, Falconiformes, Coraciiformes and Passeriformes. For monitoring the birds BRESSER 10 × 50 binoculars and an eyepiece BRESSER 20–60 telescope were used.

Statistical analysis

The data, i.e. the sums of all individuals of each species observed during summer (April to September) and winter (October to March), were analyzed using ordination methods. The aim of lumping the data for each season was to increase the explanatory power of the analysis, because the occurrence of birds differed markedly between summer and winter, but the abundances recorded at each observation were usually relatively low and would therefore not meet the prerequisites for the use of multivariate methods.

In the direct gradient analysis CCA in the program CANOCO for Windows 4.5 the following environmental variables were used: type of watery locality, total depth of the reservoirs monitored, acreage of littoral vegetation at the localities monitored, diversity index, number of species of birds at the different localities and size of the fish stock. In the graph, the environmental variables selected were those that were not significant at the 5% significance level based on the MONTE CARLO permutation test. The resultant number of birds was logarithmically transformed (Statistica 2007).

Results

List of species occurring at the localities monitored

The following species of waterbirds occurred at the fishponds and sandpits from March 2005 to February 2006: little grebe (*Tachybaptus ruficollis*), great crested grebe (*Podiceps cristatus*), cormorant (*Phalacrocorax carbo*), little bittern (*Ixobrychus minutus*), night heron (*Nycticorax nycticorax*), little egret (*Egretta garzetta*), great white egret (*Egretta alba*), grey heron (*Ardea cinerea*), mute swan (*Cygnus olor*), greylag geese (*Anser anser*), mallard (*Anas platyrhynchos*), gadwall (*Anas strepera*),

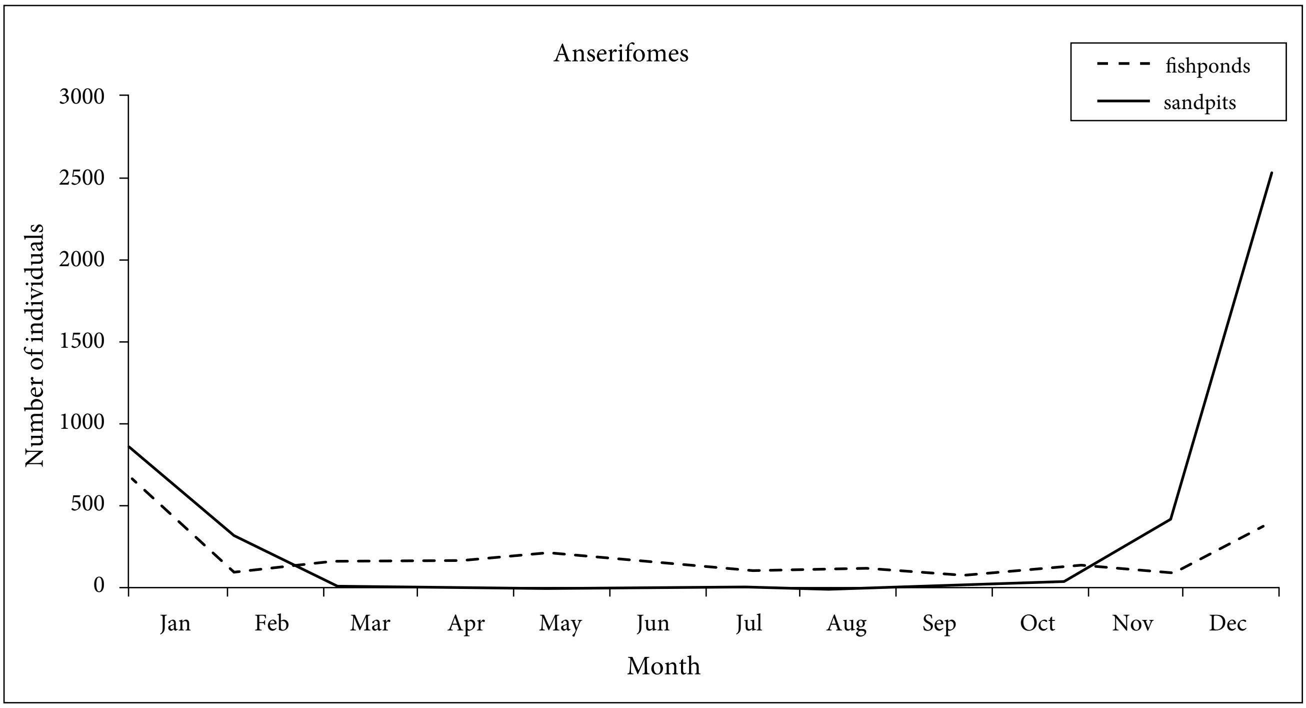

Fig. 1 Comparison of the abundance of birds of the order Anseriformes at NFS and sandpits in the Třeboň area.

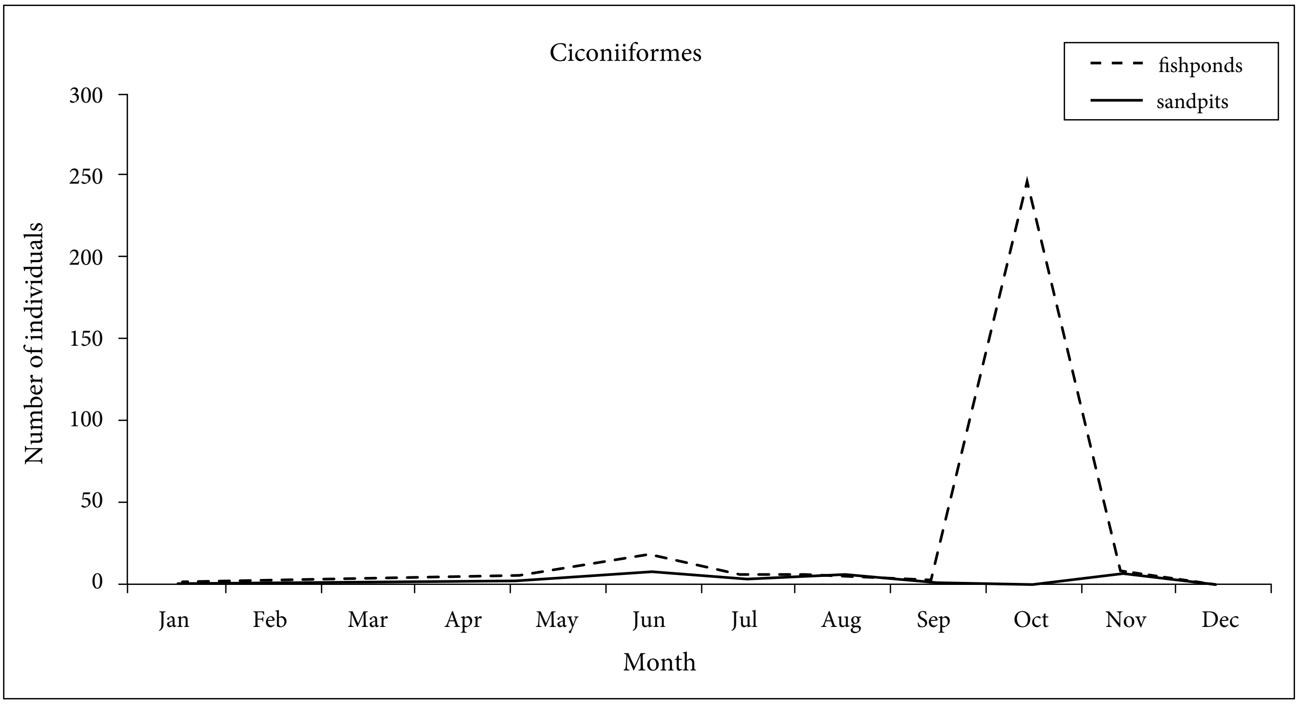

Fig. 2 Comparison of the abundance of birds of the order Ciconiiformes at NFS and sandpits in the Třeboň area.

pochard (*Aythya ferina*), tufted duck (*Aythya fuligula*), goldeneye (*Bucephala clangula*), red-crested pochard (*Netta rufina*), osprey (*Pandion haliaetus*), white-tailed eagle (*Haliaetus albicilla*), marsh harrier (*Circus aeruginosus*), moorhen (*Gallinula chloropus*), coot (*Fulica atra*), black-headed gull (*Larus ridibundus*), common gull (*Larus canus*), common tern (*Sterna hirundo*), kingfisher (*Alcedo atthis*) and sand martin (*Riparia riparia*).

Comparison of the abundance of waterbirds recorded at fishponds and sandpits

In NFS, the most frequent species were black-headed gull (*Larus ridibundus*), mallard (*Anas platyrhynchos*) and great crested grebe (*Podiceps cristatus*). Compared to the NFS, there were no markedly frequent species recorded at the Veselí sandpits: these watery areas are not very attractive for waterbirds. Also in comparison with

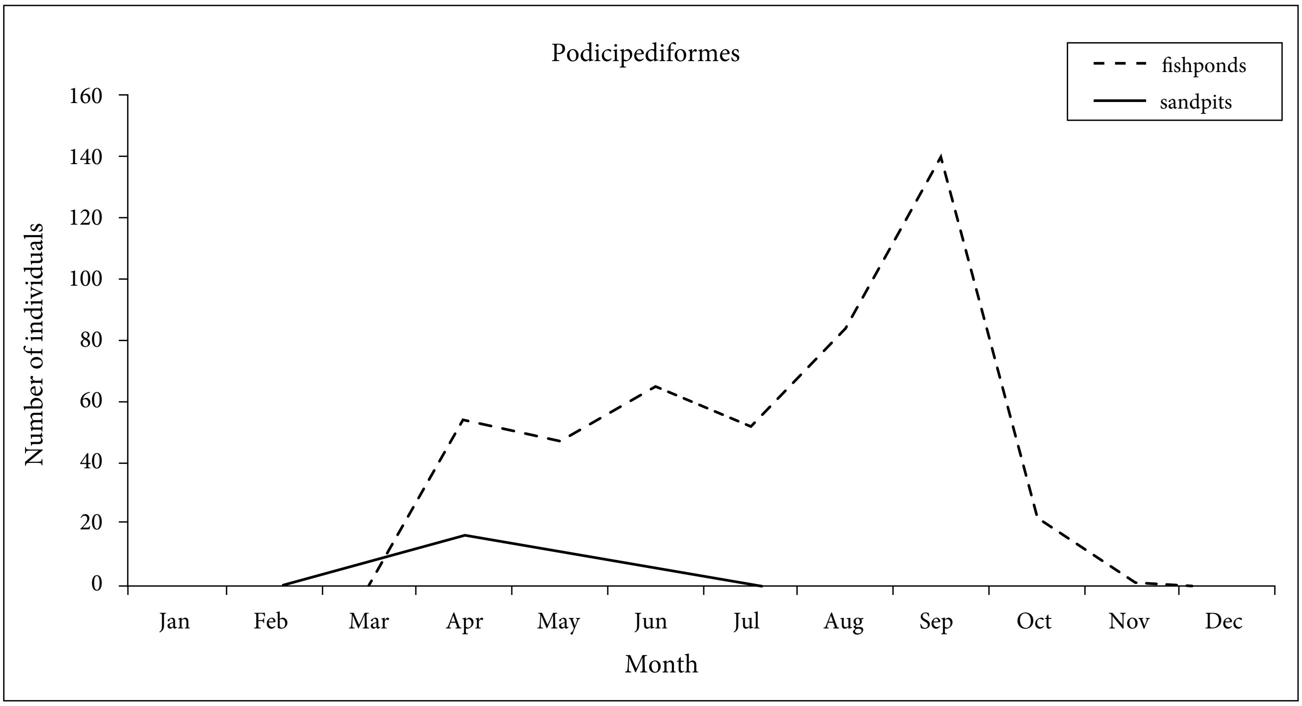

Fig. 3 Comparison of the abundance of birds of the order Podicipediformes at NFS and sandpits in the Třeboň area.

other sandpits, the Veselí sandpits are less attractive for birds. During autumn and winter, mallard (*Anas platyrhynchos*) and cormorant (*Phalacrocorax carbo*) occurred abundantly, but less frequently than at other localities.

Although at the Veselí and Cep sandpit systems waterbirds were less abundant during the nesting season, in winter the Cep sandpit system was the locality with the greatest number of wintering waterbirds, mainly mallard (*Anas platyrhynchos*). A similar trend was also recorded for the Halámky sandpit system. Waterbirds were always significantly more numerous at fishponds than nearby sandpits (Figs 1, 2, 3).

A high incidence of the following bird orders was reported in the NFS: Anseriformes – mallard (*Anas platyrhynchos*), Gadwall (*Anas strepera*), pochard (*Aythya ferina*), tufted duck (*Aythya fuligula*), red-crested pochard (*Netta rufina*), goldeneye (*Bucephala clangula*), mute swan (*Cygnus olor*) and greylag geese (*Anser anser*), followed by Podicipediformes – great crested grebe (*Podiceps cristatus*), little grebe (*Tachybaptus ruficollis*) and Charadriiformes – black-headed gull (*Larus ridibundus*) and common tern (*Sterna hirundo*). Grey heron (*Ardea cinerea*), great white egret (*Egretta alba*), little egret (*Egretta garzetta*) and night heron (*Nycticorax nycticorax*) of the order Ciconiiformes were also relatively abundant at the fishpond localities, but less so than the species listed above. Abundance of birds of this order was not significantly different from their numbers recorded at sandpits, where only the grey heron (*Ardea cinerea*) was recorded. Bird abundance recorded in October, which

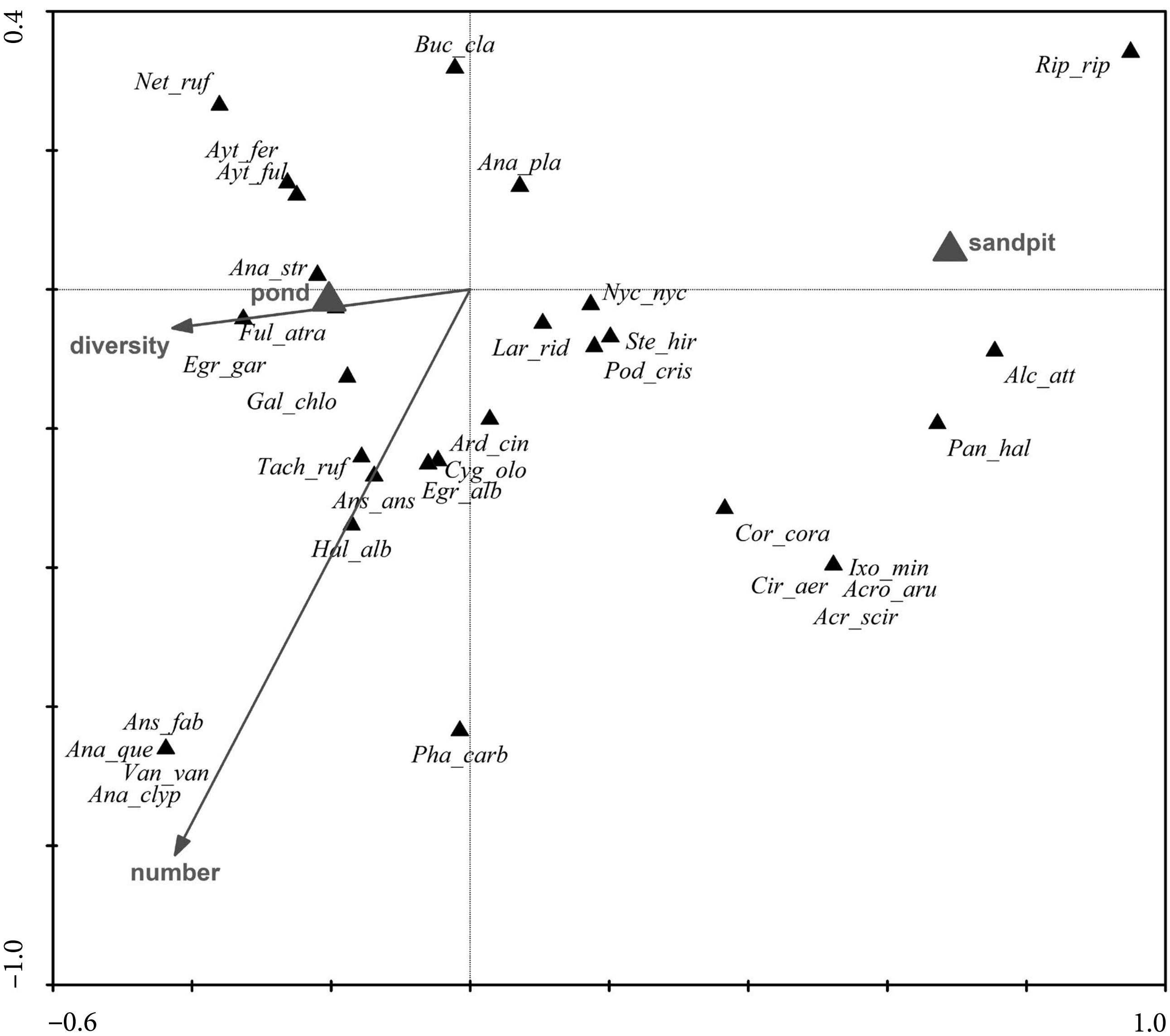

Fig. 4 Influence of environmental variables on the occurrence and abundance of waterbirds at watery localities in summer. Notation used: ***Net_ruf*** – Red-Crested Pochard, ***Ayt_ful*** – Tufted Duck, ***Ayt_fer*** – Pochard, ***Ana_str*** –Gadwall, ***Ful_atra*** – Coot, ***Egr_gar*** – Little Egret, ***Ans_ans*** – Greylag, ***Gal_chlo*** – Moorhen, ***Tach_ruf*** – Little Grebe, ***Cyg_olo*** – Mute Swan, ***Egr_alb*** – Great White Egret, ***Hal_alb*** – White-tailed Eagle, ***Pha_carb*** – Cormorant, ***Buc_cla*** – Goldeneye, ***Ana_pla*** – Mallard, ***Nyc_nyc*** – Night Heron, ***Ste_hir*** – Common Tern, ***Pod_cris*** – Great Crested Grebe, ***Lar_rid*** – Black-headed Gull, ***Ard_cin*** – Grey Heron, ***Cor_cora*** – Raven, ***Cir_aer*** – Marsh Harrier, ***Acro_aru*** – Grear Reed Warbler, ***Acro_scir*** – Reed Warbler, ***Ixo_min*** – Little Bittern, ***Pan_hal*** – Osprey, ***Alc_att*** – Kingfisher, ***Rip_rip*** – Sand Martin.

differed from the usual abundance of birds of the order Ciconiiformes during the nesting and migration season at NFS, was influenced by the autumn release and fish harvesting at the Naděje fishpond.

Sandpits showed the opposite trend. During the nesting period, waterbirds were not very abundant there. Mallard (*Anas platyrhynchos*) was the dominant species, followed by much smaller numbers of gadwall (*Anas strepera*) and mute swan (*Cygnus olor*). Great crested grebe (*Podiceps cristatus*) and grey heron (*Ardea cinerea*) occurred only in small numbers or sporadically in sandpits. No other species of this order were observed there. In winter, waterbirds in sandpits were quite numerous, but represented by only a few species. The dominant species was again mallard (*Anas platyrhynchos*), whose abundance reached more than two thousand individuals during this period.

Figure 4 shows CANOCO analysis of the total numbers of species of birds recorded in summer. The first axis explained 14.2% of the total variability, the first and second axis together explained 22.2% of the total variability, while for the indirect analysis (without the use of environmental variables) this percentage was 23.4%.

Type of locality (fishpond/sandpit), diversity and number of species were used as the environmental variables and all of them were significant at the 5% significance level. This figure shows a higher preference for sandpits by sand martin (*Riparia riparia*), kingfisher (*Alcedo atthis*), osprey (*Pandion haliaetus*) and little bittern (*Ixobrychus minutus*). In contrast, fishponds are more attractive for red-crested pochard (*Netta rufina*), pochard (*Aythya ferina*), tufted duck (*Aythya fuligula*), greylag geese (*Anser anser*), coot (*Fulica atra*), moorhen (*Gallinula chloropus*), little grebe (*Tachybaptus ruficollis*), white-tailed eagle (*Haliaetus albicilla*) and little egret (*Egretta garzetta*). Species such as grey heron (*Ardea cinerea*), great white egret (*Egretta alba*), mute swan (*Cygnus olor*), black-headed gull (*Larus ridibundus*), common tern (*Sterna hirundo*), night heron (*Nycticorax nycticorax*), great crested grebe (*Podiceps cristatus*), mallard (*Anas platyrhynchos*), goldeneye (*Bucephala clangula*) and cormorant (*Phalacrocorax carbo*) did not show a significant preference for any type of locality.

Figure 4 also shows, which species were capable of reducing the abundance of other species of waterbirds in their vicinity by forming more numerous flocks. Species such as red-crested pochard (*Netta rufina*), pochard (*Aythya ferina*), tufted duck (*Aythya fuligula*) and gadwall (*Anas strepera*) formed less numerous flocks, which included other species.

Discussion

The total abundance of waterbirds at the fishponds monitored over the period 1986–2000 by (Ševčík 2005) was slightly different from that reported in this study. However, the numbers of waterbirds that might be indicative of sudden changes in the factors influencing their occurrence did not change significantly at these fishponds. Data provided by the Administration of Třeboňsko PLA are relatively consistent with those presented here.

Older results (Balounová et al. 1996; Pešata 2003) indicate small numbers of waterbirds present at small fishponds (up to 5 hectares). The recent increase in the preference for smaller fishponds reported here may be influenced by food supply or the amount and localization of littoral vegetation, as the littoral vegetation provides shelter and building material for nests.

Another factor influencing the preference for fishponds may be the depth of the water, as birds are better able to access food in shallow water. A negative factor that can influence nesting success at these localities is human manipulation of the water level (and depth). The influence of this was not studied in detail, but the results obtained during 2005–2006 indicate that slight changes in water level can influence the different preferences of waterbirds for fishponds and sandpits during the nesting season.

Unlike fishponds, sandpits are more attractive in autumn and winter for those waterfowl that are migrating or overwintering. Larger areas of sandpits are suitable locations for aggregating prior to migration. Also, shooting is prohibited close to sandpits and therefore these reservoirs serve as refuges for birds during the hunting season. The most important factor, however, is the water in sandpits is free of ice during winter due to sand mining. The lower attractiveness of sandpits during the nesting season is due the fewer sources of food there and greater depth of water, which prevents some species of waterbirds from accessing food and the absence or only small areas of littoral vegetation resulting in fewer potential nesting sites for many species. The disturbing factor is the number of visitors, especially holidaymakers and fishermen.

Conclusions

Waterbirds prefer fishponds to sandpits during the nesting period because more food is available in fishponds for feeding to the young. Another factor is probably the occurrence there of more littoral vegetation. Sandpits are more attractive for waterbirds at other times of the year when they are mainly visited by some species during migration and for overwintering. Both of these habitats are important biotopes for waterbirds in the Protected Landscape Area (PLA), Biosphere Reserve and Important Bird Area (IBA) Třeboňsko.

Acknowledgements

Final processing of the results of this study was funded by project CZ.1.07/2.3.00/09.0076.

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PRESERVATION OF WILDERNESS AREAS IN EUROPE

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ABSTRACT

A unique momentum has been created over the past few years for strengthening the protection of wilderness in Europe. Policy makers started to pay attention to the importance of truly untouched and non-managed areas and the European Parliament adopted a special report on wilderness in February 2009. The report was followed by the EC Presidency Conference in Prague, May 2009, on Wilderness Areas. The most important outcome of this event was the approval of the 'Agenda for Wilderness', which eventually led to the inclusion of wilderness in the new EU Biodiversity Strategy. This paper argues that these political successes have yet to be put into practice. Threats to wilderness areas are still increasing and there have been no improvements in the management of these areas. There are emerging threats, especially from tree felling and mining, which is driven by increase in commodity prices. In order to save the last pieces of wilderness in Europe and utilize the current opportunities to restore wilderness areas, science and field conservation must develop a common Wilderness Research Agenda for Europe. The main questions are: (i) What are the ecosystem services and benefits that humans obtain for wilderness areas? (ii) What is the potential contribution of such wilderness areas for reducing biodiversity loss, halt species extinctions and support biodiversity restoration in Europe? (iii) What is the social perception of wilderness in different countries and across different sectors of society? (iv) What should be considered wilderness in a densely populated area such as Europe?

Keywords: wilderness, Europe, biodiversity, mountains, restoration

Background

Discussions on the new European biodiversity strategy and inclusion of wilderness in a European conservation vision was first suggested during the 1st European Conference on Conservation Biology (ECCB) in Eger in 2006. It was argued that 'natural processes that favour biodiversity and provide ecosystem services, including e.g. the need to define wilderness areas in some close to climax habitats, need to be addressed' (Miko 2006). This seminal presentation resulted in a discussion involving the European Commission, scientific and civil society sectors. Following on the conference in Eger, a seminar on wilderness was organised during the 3rd ECCB in Glasgow. This seminar focused not only on aspects linked to conservation biology, but adopted a multidisciplinary approach and included aspects of both social and geographic studies. During this seminar there was an offer to dedicate a special issue of the European Journal of Environmental Sciences to European wilderness areas. This paper is also a unique opportunity to promote the Science symposium of the 10th World Wilderness Congress, which will be held in Salamanca, Spain, in October 2013.

There has been an move to increase the protection of wilderness areas in Europe over the past 5 years. While the protection of wilderness areas has a long history in the US starting with the approval of the US Wilderness Act in 1964, this is a relatively new phenomenon in Europe.

Protected areas in Europe

Territorial protection in Europe started with the designation of the Abisko National Park in 1909 and boosted in the 1970s when IUCN introduced the concept of landscape protected areas. The Category V, Landscape Protected Area, opened up the opportunity to protect larger modified landscapes and rural areas with the aim of maintaining biodiversity. One can argue that the value of such highly modified landscapes, although important for protecting certain species, is not necessarily mainly the maintenance of biodiversity. They are still used for producing various agriculture or forest products and subject to constant human intervention.

For instance several participants at the Euromontana conference in Lillehammer in 2010 argued for increasing agriculture related subsidies in order to maintain the traditional land use practices in the mountains of Europe. The argument was mainly linked to extensive farming (Kun 2011). People argued that most mountain grasslands are secondary vegetation formations whose continuity requires a certain level of human maintenance. Natural processes as a potential way of maintaining and strengthening the protection of biodiversity are still largely ignored in Europe.

The boom in using IUCN categories for modified landscape protection resulted in a huge imbalance between the representation of modified landscapes and wilderness areas in Europe. A recent report of the European Environmental Agency (EEA) reports that the area protected in the 39 EEA countries is around 21% (EEA 2012), whereas that of wilderness is around 1% (European Parliament 2009). However, there is no scientific evidence for this figure!

These two numbers, 21% protected compared to 1% wilderness, demonstrates the lack of wilderness and non-intervention management in Europe. However there are some important steps towards strengthening the protection of wilderness areas in Europe

Chronology of wilderness policy development

In 2008 over 100 organisations representing various interests from the civil society sector to tourism and governments signed a Resolution on Wilderness and submitted the document to the European Commission requesting stronger protection of wilderness areas.

Following upon this resolution, the European Parliament adopted a special report on wilderness on 3 February 2009. This report was supported by an overwhelming majority of the MEPs with over 500 voting yes and only 19 voting no. This report requested the European Commission to take further concrete steps for the better preservation of wilderness areas.

In order to define these concrete steps the European Commission financed a Conference on Wilderness and Wild Land in Europe during the Czech presidency of the EC (Prague, May 2009). The most important outcome from this conference was the adoption of the Agenda for Europe's Wilderness, which lists 24 recommendations covering the following 4 fields: policy development, awareness raising, generating additional information and developing a supporting capacity.

After this conference it was very obvious that there was a need for much additional work on two aspects of wilderness protection: enforcement of the protection of existing wilderness areas and restoration of areas to wilderness in order to increase the coverage of wilderness.

The 3rd Global Biodiversity Outlook report mentions the opportunity for "rewilding landscapes from farmland abandonment in some regions – particularly in Europe, where about 200,000 square kilometers of land are expected to be freed up by 2050. Ecological restoration and reintroduction of large herbivores and carnivores will be important in creating self-sustaining ecosystems with minimal need for further human intervention." (CBD 2010). The expectation that it is possible to restore a large proportion of land to wilderness areas is of great importance for rural regions in EU member states. Land abandonment is especially important in mountainous areas (IEEP 2011).

In terms of wilderness, the greatest area in Europe is located in the Nordic mountains. Elsewhere, only Spain has more than 10,000 km^2 of mountain wilderness. Of the total area designated as Natura 2000 sites, 43% is in mountainous areas, compared to 29% for the EU as a whole. These sites cover 14% of the mountainous area in the EU (EEA 2010).

In order to exploit the opportunities for restoring wilderness areas in Europe, a conference was organised under the Belgium EC Presidency in November 2010, which was specifically dedicated to restoring wilderness areas (entitled Restoring the Wild Heart of Europe). Following upon this conference the European Commission published a special guidance document on wilderness for Natura 2000 site managers. Although this guidance document has yet to be approved, the draft version, which is currently under review, is available on the DG Environment's website in the wilderness part of the Natura 2000 section (European Commission 2012).

Historic opportunity

There is now a great opportunity to set up a European Wilderness Preservation System within the framework of the network of existing protected areas and Natura 2000. The European Commission initiated a new project to develop an online database of wilderness areas. This register is to be finalised by June 2013 and will be an open database, which will reveal the actual coverage of wilderness in 39 European countries.

This register will help to identify those areas that can be categorized as protected wilderness. Hopefully it will also indicate opportunities for increasing the coverage of wilderness areas by slight changes in management of existing protected areas. The database may also highlight existing and possible future threats to wilderness areas.

This register will provide many opportunities and also a strong basis for arriving at an acceptable definition of wilderness. The Europarc Federation established a working group in August 2009 with the aim to develop a commonly accepted definition of wilderness for Europe. This working group recently completed this task and the European Commission agreed with its recommendation that the following definition be adopted:

A wilderness is an area governed by natural processes. It is composed of native habitats and species, and large enough for the effective ecological functioning of natural processes. It is unmodified or only slightly modified and without intrusive or extractive human activity, settlements, infrastructure or visual disturbance.

This definition clearly indicates that wilderness is not a buzzword or another word for biodiversity.

Threats to wilderness

Ironically there are opportunities for wilderness to exist in Europe in spite of increasing threats to their existence. Despite the ambitious target adopted by the world's governments of reducing the rate of loss of biodiversity by 2010, biodiversity continues to decline (Butchart et al. 2010) and scenarios for the future indicate that the window of opportunity for reversing biodiversity loss is closing (Pereira et al. 2010). After failing to meet the 2010 biodiversity target, the parties to the Convention on Biological Diversity have just agreed in Nagoya a new set of ambitious targets for 2020. These targets include for example to increase the coverage of protected areas from 12% of the land surface to 17% and ensure that all areas under forestry are managed sustainably in the future.

Wilderness areas are core zones of Europe's Green infrastructure and are likely to play a significant role in reducing the loss of biodiversity. However, there is increasing pressure on these areas throughout Europe. The major threats to wilderness are:

1. The tendency to regard 'traditional' land-use as a way of maintaining the current European landscape;
2. Over grazing by domestic and semi-domestic breeds, which are used as a replacement for wild grazers;
3. Mining: the increasing commodity prices put a high pressure on wild areas, and the extraction of timber (e.g. in Czech Republic & the Carpathians);
4. Energy projects that aim to develop more hydropower (e.g. in Turkey), wind farms (eg. in Scotland) and biofuel as a way of utilizing marginal farmland areas;
5. Development of unsustainable tourism projects like new ski resorts in the Sumava or Balkan mountains.

There is also an increasing tendency in Europe to use semi-domestic herbivores to mimic natural grazing. These projects try to achieve an abundance of wildlife equivalent to that in wilderness areas. Their main argument in support of this is the concern over the loss of biodiversity attributable to the decrease in grazing pressure. However these projects do not take into account that natural grazing pressure was very likely much lower in Europe than in extensive farmland areas. The decline in Spanish dehesas is an interesting example of the consequence of an increase in the demand for meat products.

How to proceed

As Ladislav Miko argued for a new vision of nature conservation for Europe at Eger in 2006 this paper argues for a vision of wilderness for Europe. In addition to the moral argument there is also the financial incentive of linking it with payment for ecosystem services.

According to the EEA database on land use in Europe, over 4% of the land on this continent is already covered with artificial surfaces (infrastructure, housing and industry) and this increased annually by over 110,000 ha between 2000 and 2006, and is likely to increase in the future.

This means that soon the cover by such surfaces will be roughly 5% of Europe's land area, which must be compensated for by having 5% where the rule of natural processes prevail. That is the area included in the European Wilderness Preservation System should be at least equivalent to 5% of the land area. This does not require additional area but can and must be achieved within the current 21% protected areas.

Finally there are four major recommendations for improving the protection of wilderness areas in Europe and using the opportunities these afford to counter the threats:

1. the commonly agreed definition of wilderness must be used throughout the continent
2. the reintroduction of artificial substitutes for extinct species should not be claimed as wilderness restoration
3. focus on strict protection of those wilderness areas that still exist in Europe that can be used as role models for restoration projects
4. focus on educating professionals and developing a mass communication campaign for wilderness in Europe

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CAN NATURA 2000 MAPPING BE USED TO ZONE THE ŠUMAVA NATIONAL PARK?

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ABSTRACT

The future of the Šumava National Park is linked to discussions on its zoning, which has undergone significant changes since the establishment of the Park. The present zonation was strongly criticized by experts, representatives of NGOs and international organizations. Over the last couple of years, politicians became more vocal in callings for a new law that would fix the rules governing the use of the Šumava NP and the central question in this is zonation. A new proposal on the zonation was prepared by the scientific community, but in order to maximize the probability of its acceptance, the area of the core zone proposed was just barely sufficient to include the most valuable parts of the most important habitats and some of the habitats of the most important species. Thus, any further reduction in the area of the core zone is likely to pose a serious threat to the survival of important species and habitats occurring in the Šumava NP. Here we present a politically unbiased estimate of the optimum size of the core zone based on Natura 2000 habitats and species mapping.

Keywords: zonation, management of national park, biodiversity, Natura 2000, GIS

Introduction

The Bohemian Forest (Šumava Mts. in Czech) is a mountain ridge along the Czech-Bavarian border in the heart of Europe. This densely wooded landscape, comprising crystal clear mountain streams, unspoilt marshlands, mires and bog woodlands, and abandoned mountain pastures at high altitudes, is a refuge for many endangered species of plants and animals. Elements of northern boreal forest represented here include capercaillie (*Tetrao urogallus*), Ural owl (*Strix uralensis*) and three-toed woodpecker (*Picoides tridactylus*). This transboundary area is also home to several iconic species, such as lynx (*Lynx lynx*), moose (*Alces alces*), peregrine (*Falco* peregrinus) and freshwater pearl mussel (*Margaritifera margaritifera*), each of which now occurs only in few viable populations in Central Europe. Nature is protected here in two national parks (Fig. 1): the Bayerischer Wald NP (Germany; established 1969 and enlarged in 1997 to 24,250 ha) and the Šumava NP (Czech Republic; established 1991; 68,064 ha). They constitute the largest cross-border protected area in Central Europe (Křenová and Kiener 2012). Each component is the largest terrestrial Natura 2000 site in the respective countries and a significant part of the Natura 2000 network, which was established to protect the most endangered habitats and species in Europe, as defined in the 1992 Habitats Directive and 1979 Birds Directive.

Formally, the Natura 2000 network recognizes *Sites of Community Importance* (SCI), in terms of 231 habitat types and 911 animal and plant species defined in the Habitats Directive, and *Special Protection Areas* (SPA), in terms of 194 species of bird defined in the Bird Directive (Sunseth and Creed 2010). The **SCI Šumava**, the largest in the Czech Republic, was designated by Government Order No. 132/2005. It is a unique mosaic of natural and secondary habitats of exceptional natural value of European-wide significance. Each type of habitat hosts numerous rare and protected plant and animal species. The **SAC Šumava** was designated by Government Order No. 681/2004 on December 8, 2004. Among the many species in Annex I of the Birds Directive that occur here, the most notable are three species of grouse, especially capercaillie (*Tetrao urogallus*). Among the handful of populations of capercaillie still persisting in highland ecosystems in Central Europe, the population in the Bohemian Forest now represents the only viable one in the Czech Republic.

For a long time now the debates about the future of nature conservation in the Šumava NP have involved discussions on the zoning of the Park, which has undergone significant changes since its establishment (Křenová and Hruška 2012). When the NP was founded in 1991, its zonation was based on internationally accepted concepts. Change in the leadership of the national park in 1995 brought about a change in management. Zone I, the most valuable and strictly protected part of the NP (equivalent to the core zone under Czech legislation), was reduced in size and the original 54 units were further fragmented into 135 smaller units. The main reason for this was a strong desire for active management, mainly logging of bark beetle-infested trees. This was strongly criticized by experts, representatives of NGOs and international organizations (IUCN, Ramsar Committee).

During 2004–2005, a new zonation was proposed by scientists and Šumava NP Authorities, which included the recommendations of experts, but unfortunately was not officially approved, because of opposition from local communities and politicians. During the last couple of years, politicians became more vocal in calling for a new law that would fix the rules governing the use of the Šumava NP and the central question in this is the zonation of the Park.

A new proposal for the zonation of the Park was prepared by a scientific committee (Křenová and Hruška 2012) and submitted to the Ministry of Environment. This proposal took into account the current condition of the Šumava ecosystems, the latest knowledge and understanding of their dynamics, and the effects of natural disturbances. A special emphasis was placed on the population dynamics and ecological requirements of flagship species, such as capercaillie. To maximize the probability of acceptance of this proposal by the politicians, the strictly protected Zone I is only just large enough to include the most valuable parts of the most important habitats and some of the locations where the most important species occur. Thus, the scientific proposal is the minimum acceptable as any further reduction in Zone I is likely to seriously threaten the survival of certain important species and habitats in the Šumava NP. However, we feel that the scientific community should be aware that it is possible to arrive at a politically unbiased proposal, which defines the *optimum* size of the core zone of the Šumava NP and does not pander to the views of local politicians. Here we present such a proposal, which is based on data from Natura 2000 habitats and species mapping.

Methods

Natura 2000 data

Mapping of habitats organized by the Nature Conservation Agency of the Czech Republic (NCA) and that of important bird areas, organized by the NCA in cooperation with the Czech Ornithological Society, the Czech partner of the Bird Life International, is an extensive and ambitious project set up as part of the Natura 2000 network in this country (Hartel et al. 2009). Pilot habitat mapping was carried out in 2000. Mapping was carried out during 2001-2004 at two levels: *detailed mapping* focused on areas with an abundant occurrence of natural habitats and selective mapping (*contextual mapping*) in the rest of the country. In addition to the indication of a habitat on a map, data on its representativeness, conservation status and other characteristics were also recorded. More details are available on the methodology of habitat mapping in Guth (2002). The Šumava NP Headquarters was deeply involved in the preparation of the Natura 2000 network. There was also a bilateral project with the Bayerischer Forest NP, which resulted in the publication of a trans-boundary map of Natura 2000 habitats and species locations (Hußlein and Kiener 2007), and joint discussions on the appropriate management practices for Natura 2000 sites (Hußlein et al. 2009).

SCI Šumava (171,959 ha) is the largest SCI in the Czech Republic and includes the whole of the Šumava NP, parts of the Šumava Protected Landscape Area and the Šumava Biosphere Reserve. Because of its great importance for nature conservation the whole of the Šumava NP was mapped in detail. The habitat mapping followed the methodology of Chytrý et al. (2001) and was carried out by botanists. Digitized data from this mapping project was used to define the area in terms of the Natura 2000 network (natural habitat types) and in the management of this protected area. Nineteen habitats in the Annex I and ten species in Annex II of the Habitats Directive are protected in the Šumava NP (Table 1). The most valuable habitats include the well-preserved complex of peat and wetland habitats, primeval forests and species-rich secondary mountain grasslands.

It is known that non-intervention management of protected areas and large Natura 2000 sites significantly improves habitat quality and living conditions for species depending on natural dynamic processes (e.g. all primary forest habitats in Central Europe) and therefore provides a substantial contribution to maintaining current levels of biodiversity (Müller et al. 2008; Lehnert et al. 2013). Non-intervention management should thus focus mainly on primary habitats and large areas with the capacity of self-restoration (Hußlein et al. 2009).

Therefore, the following four habitats protected in SCI Šumava were selected as basic components of the non-intervention parts of Zone I in the Šumava NP:

1. ***spruce forests*** (L9.1 Montane *Calamagrostis* spruce forests, L9.2A Bog spruce forests, L9.2B Water logged spruce forests, L9.3 Montane *Athyriu*m spruce forests),
2. ***beech forests*** (L5.1 Herb-rich beech forests, L5.4 Acidophilous beech forests),
3. ***raised bogs, springs, fens and transitional mires*** (R2.2 Acidic moss-rich fens, R2.3 Transitional mires, R3.1 Open raised bogs, R3.2 Raised bogs with *Pinus mugo*, R3.3 Bog hollows),
4. ***other valuable NATURA 2000 habitats*** – usually mosaics of the following habitats: A4.2 Subalpine tall-forb vegetation, A4.3 Subalpine tall-fern vegetation, L2.1 Montane grey alder galleries, L2.2 Ash-alder alluvial forests, L4 Ravine forests, L10.1 Birch mire forests, L10.2 Pine mire forests with *Vaccinium* and L10.4 *Pinus rotundata* bog forests which occur usually in mosaic with other spruce or beech habitats mentioned above. Other habitats such as V1 Macrophyte vegetation of naturally eutrophic and mesotrophic still waters occur in/along several rivers in NP. Last group of valuable habitats is represented by semi-natural meadows and pastures, which consist of T1.2 Montane *Trisetum* meadows, T1.6 Wet *Filipendula* grasslands, T1.9

Table 1 Habitats and species which are subject to protection in SAC and SCI Šumava. Priority habitats and species are marked by *.

Code	Habitats of Annex I of Habitat Directives	(ha)	Biotop units for mapping (*see Chytrý et al. 2001*)
3130	Oligotrophic to mesotrophic standing waters with vegetation of the *Littorelletea uniflorae* and/or of the *Isoëto-Nanojuncetea*	27.38	M2.2 – Annual vegetation on wet sands M3 – Vegetation of perennial amphibious herbs V6 – *Isöetes* vegetation
3150	Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition* – type vegetation	16.98	V1 – Macrophyte vegetation of naturally eutrophic and mesotrophic still waters
3260	Water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion* vegetation	81.99	V4A – Macrophyte vegetation of water streams with currently present aquatic macrophytes
4030	European dry heaths	84.48	T8.2B – Secondary submontane and montane heaths without *Juniperus communis*
5130	*Juniperus communis* formations on heaths or calcareous grasslands	5.07	T8.2A – Secondary submontane and montane heaths with *Juniperus communis*
6230*	Species-rich *Nardus* grasslands, on siliceous substrates in mountain areas (and submountain areas, in Continental Europe)	1066.89	T2.1 – Subalpine *Nardus* meadows T2.3B – Submontane or montane *Nardus* meadows without *Juniperus communis*
6410	*Molinia* meadows on calcareous, peaty or clayey-siltladen soils (*Molinion caeruleae*)	221.66	T1.9 – Intermittently wet *Molinia* meadows
6430	Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels	545.13	A4.2 – Subalpine tall-forb vegetation A4.3 – Subalpine tall-fern vegetation T1.6 – Wet *Filipendula* grasslands
6510	Lowland hay meadows (*Alopecurus pratensis*, *Sanguisorba officinalis*)	579.06	T1.1 – Mesic *Arrhenatherum* meadows
6520	Mountain hay meadows	2997.42	T1.2 – Montane *Trisetum* meadows
7110*	Active raised bogs	383.86	R3.1 – Open rised bogs R3.3 – Bog hollows
7140	Transition mires and quaking bogs	1255.29	R2.2 – Acidic moss-rich fens R2.3 – Transition mires
8220	Siliceous rocky slopes with chasmophytic vegetation	167.83	S1.2 – Chasmophytic vegetation of siliceous cliffs and boulder screes A6B – Acidophilous vegetation of alpine cliffs
9110	*Luzulo-Fagetum* beech forests	15966.51	L5.4 – Acidophilous beech forests
9130	*Asperulo-Fagetum* beech forests	2092.32	L5.1 – Herb-rich beech forests
9180*	*Tilio-Acerion* forests of slopes, screes and ravines	136.76	L4 – Ravine forests
91D0*	Bog woodland	3566.74	L9.2A – Bog spruce forests L10.1 – Birch mire forests L10.2 – Pine mire forests with *Vaccinium* L10.4 – *Pinus rotundata* bog forests R3.2 – Raised bogs with *Pinus mugo*
91E0*	Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion, Alnion incanae, Salicion albae*)	582.22	L2.1 – Montane grey alder galleries L2.2 – Ash-alder alluvial forest
9410	Acidophilous *Picea* forests of the montane to alpine levels (*Vaccinio-Piceetea*)	18567.31	L9.1 – Montane Calamagrostis spruce forests L9.2B – Waterlogged spruce forests L9.3 – Montane Anthyrium spruce forests

	Species of Annex II of the Habitat Directive
1096	*Lampetra planeri*
1324	*Myotis myotis*
1029	*Margaritifera margaritifera*
1361	*Lynx lynx*
1914*	*Carabus menetriesi pacholei*
1163	*Cottus gobio*
1303	*Rhinolophus hipposideros*
1355	*Lutra lutra*
4094*	*Gentianella bohemica*
1393	*Drepanocladus vernicosus*

	Species of Annex I of the Bird Directive
	Ciconia nigra
	Dryocopus martius
	Crex crex
	Picoides tridactylus
	Bonasa bonasia
	Glaucidium passerinum
	Aegolius funereus
	Tetrao urogallus
	Tetrao tetrix

Intermittently wet *Molinia* meadows, T2.1 Subalpine *Nardus* grasslands, T2.3B Submontane and montane *Nardus* grasslands, and finally T8.2A Secondary submontane and montane heaths.

Of course, some secondary habitats (e.g. orchid meadows) or small and fragmented areas need intervention. These, if of sufficient conservation importance, were included as managed parts of Zone I.

SAC Šumava (97,493 ha) hosts a total of 27 species listed in Annex I of the Birds Directive, 9 of which are protected here (Chvátal 2009; Table 1). Of the three grouse species occurring here, the most endangered is capercaillie (*Tetrao urogallus*). Its core areas were therefore included as the fifth component.

The data from the Natura 2000 mapping includes the five important components of Zone I. Four of them were particular habitats and the fifth the core areas for capercaillie, the most important species in this region. The sixth component was river canyons with unregulated mountain rivers, representing a specific phenomenon in this Park's landscape. There are no unique Natura 2000 habitats in the river canyons, but important species, like lynx, otter and peregrine occur there. Of course, non-intervention is the best management for such areas.

GIS analyses

All habitat and environmental data mentioned above were expressed digitally and handled in the GIS environment (ESRI ArcGIS 10.1). The proposed zonation was obtained in two steps: (i) overlay and synthesis of individual components and (ii) generalization of final output.

Initially, all components were analyzed separately in order to calculate their total area or number of patches, level of fragmentation and the percentage of the NP area they cover. After that, overlay and intersection of all the components was determined and the key habitats delineated. The crucial step was generalization of this mask, which was done using expert knowledge supported by topographical and forestry maps and aerial photographs. Forest management differs markedly within and outside the NP and so does the ownership of forests. Therefore, a 500 m wide buffer zone along the interior boundary of the NP was delineated in order to protect private forests in the vicinity against bark beetle attacks from the non-intervention zone. This width is supposed to be sufficient because bark beetle attacks trees less than 500 m from the source tree (Kautz et al. 2011). Because of the trans-boundary contracts that govern the management of forests along the Czech–Austrian border, a 200 m wide actively managed buffer strip (debarking of standing trees rather than logging) was proposed. No buffer zone was proposed along the border with the NP Bayerischer Wald, where non-intervention management is applied.

Then the following corrections and local expert knowledge were incorporated in order to improve the proposal that resulted from the above analysis:

1. adjacent units in Zone I were merged if they were separated by natural habitats;
2. forests in non-intervention parts of Zone I were extended, if applicable, to include spontaneous succession forests of mixed age structure (20–70 years old) occurring in former military training areas or abandoned fields;
3. important stands of silver fir (*Abies alba*) were added to Zone I;
4. important mating areas of black grouse (*Tetrao tetrix*), species protected in SAC Šumava, were added to Zone I. Although black grouse is not a typical species of non-intervention areas, nevertheless it is extremely sensitive to disturbance by human activities;
5. buffer zones around villages were excluded from Zone I regardless of whether or not Natura 2000 habitats occur there;
6. the border of Zone I was straightened and made to follow as far as possible natural or easily visible lines (streams, roads, etc.).

Results

The component **spruce forests**, which combines data from four biotopes (L9.1 Montane *Calamagrostis* spruce forests, L9.2A Bog spruce forests, L9.2B Water logged spruce forests, L9.3 Montane *Athyrium* spruce forests), is shown in Fig. 2 and covers an area of 160 km^2. The breeding areas of many birds, including species protected in the SAC Šumava like the three-toed woodpecker (*Pi-*

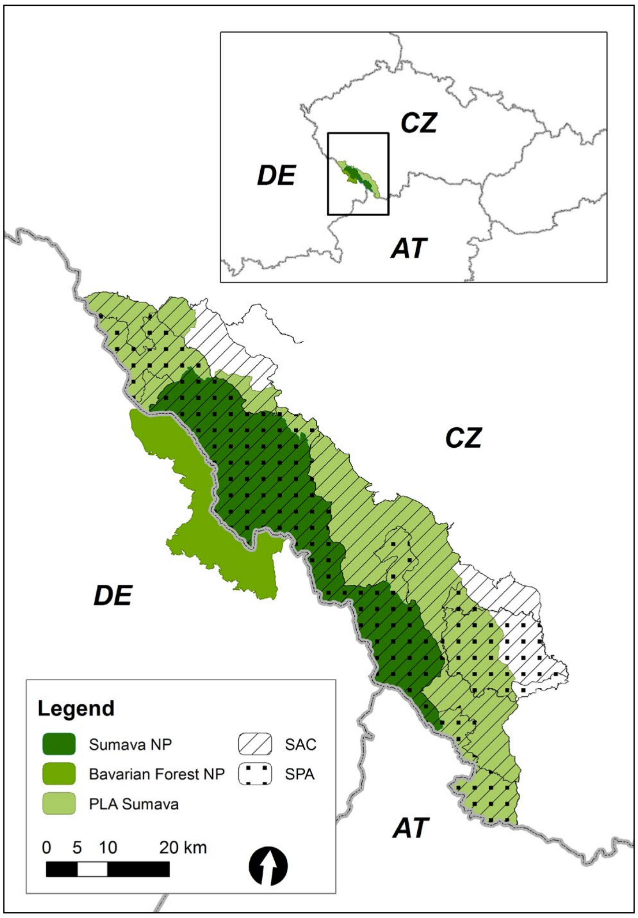


Fig. 1 Map of the Šumava National Park, Protected Landscape Area Šumava, SAC Šumava, SCI Šumava in the Czech Republic and the National Park Bayerischer Wald in Germany.

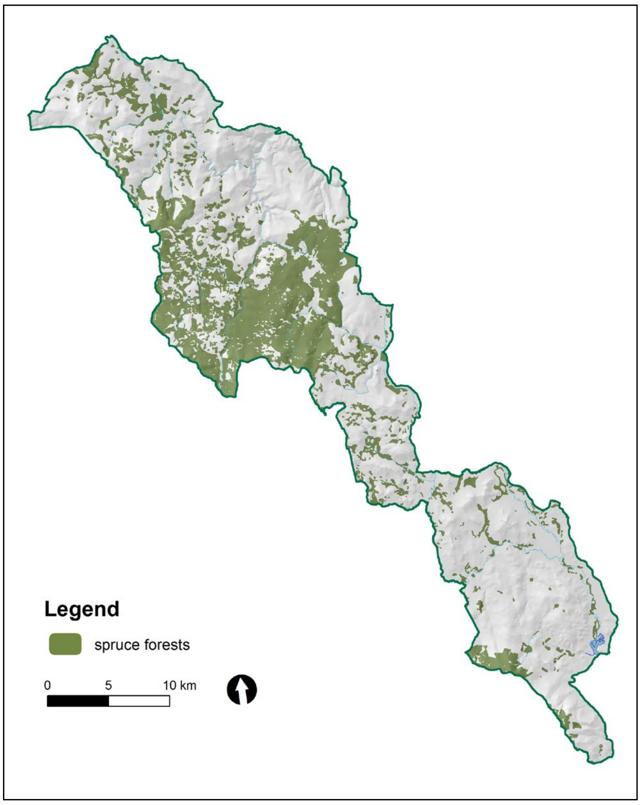


Fig. 2 Distribution of spruce forests within the Šumava NP.

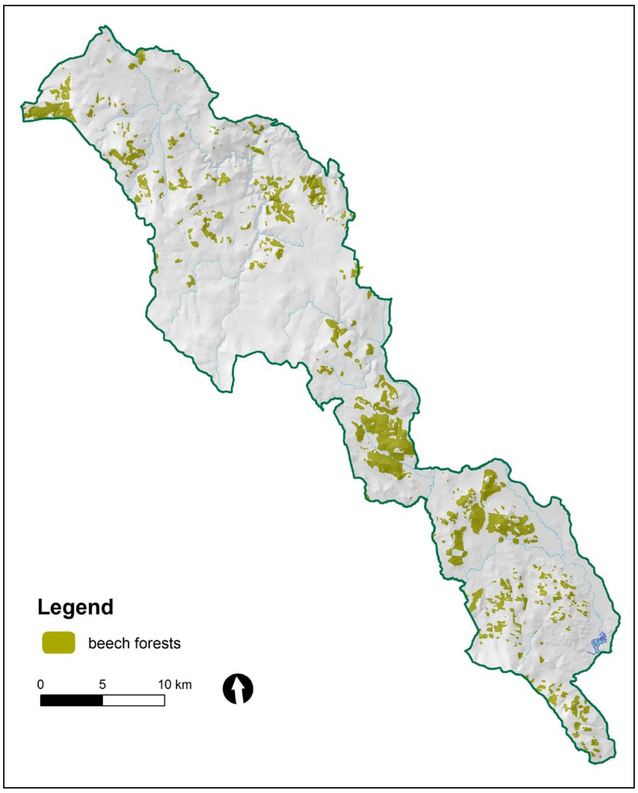


Fig. 3 Distribution of beech forests within the Šumava NP.

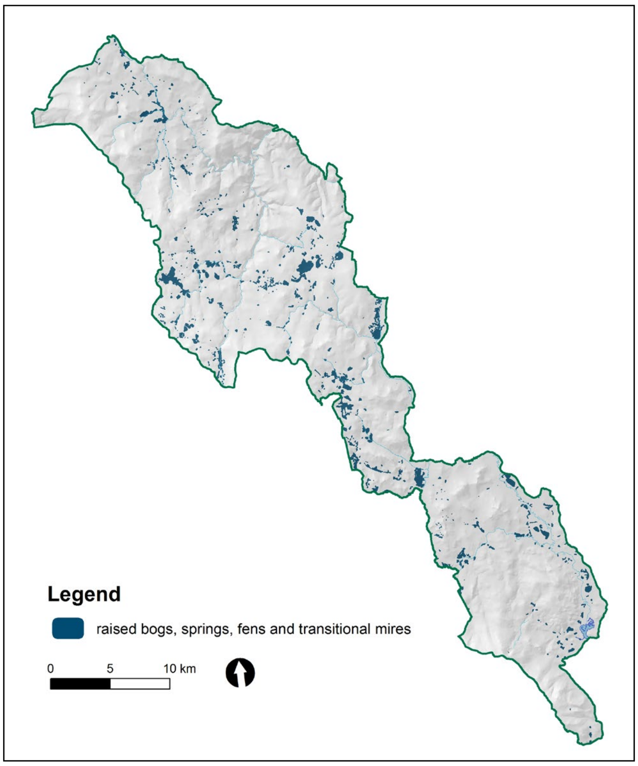


Fig. 4 Distribution of raised bogs, springs, fens and transitional mires within the Šumava NP.

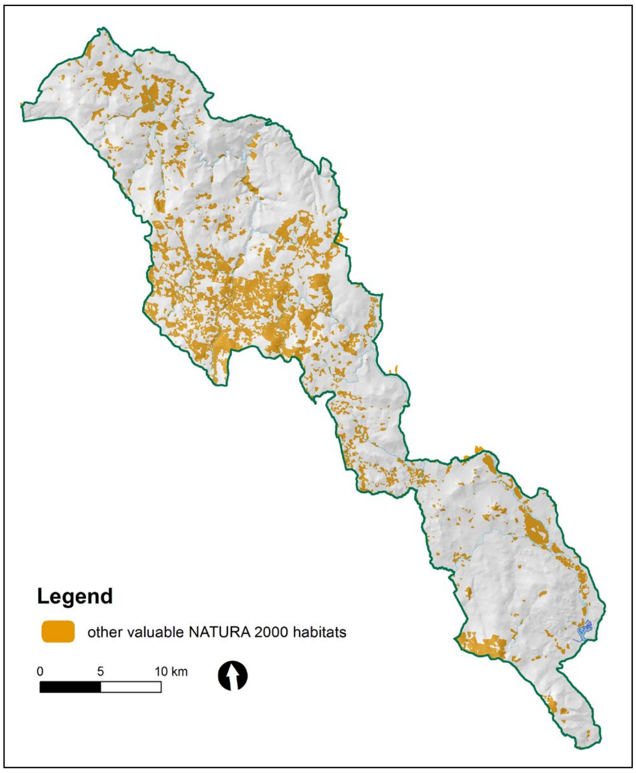


Fig. 5 Distribution of other valuable Natura 2000 biotopes within the Šumava NP.

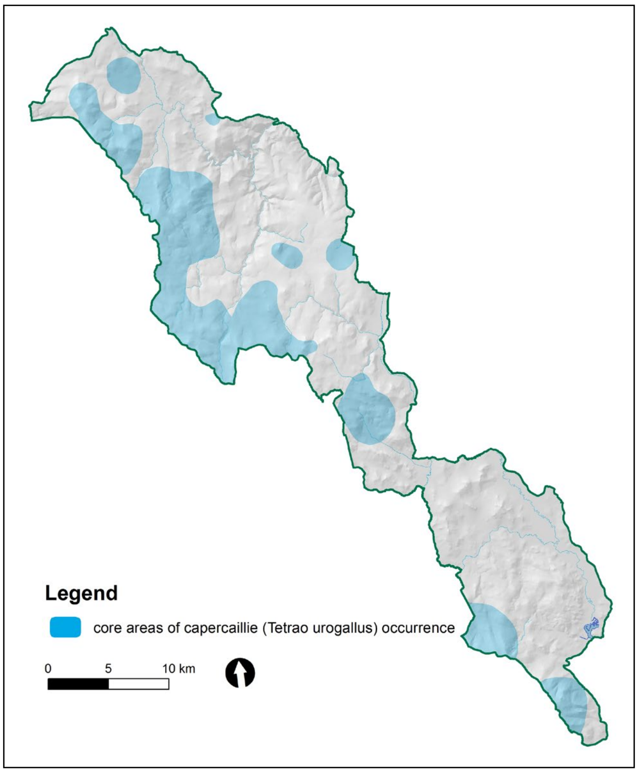


Fig. 6 Core areas of capercaillie (*Tetrao urogallus*) occurrence.

coides tridactylus), pygmy owl (*Glaucidium* passerinum) and tengmalm's owl (*Aegolius funereus*) mainly occur in this component.

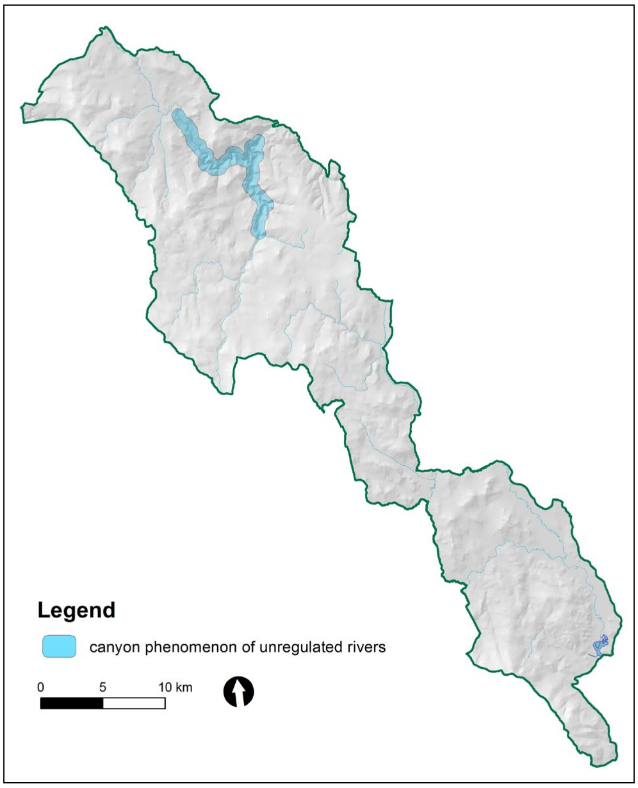


Fig. 7 River canyons phenomenon within the Šumava NP.

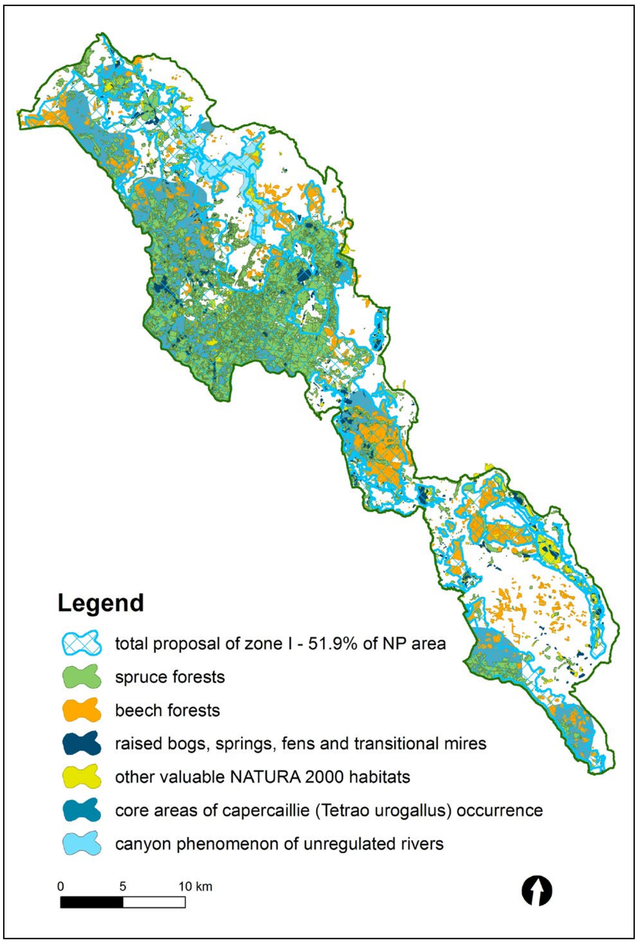
Beech forests component (Fig. 3), which combines data from two biotopes (L5.1 Herb-rich beech forests, L5.4 Acidophilous beech forests) and covers an area of 63 km^2. Only the most representative segments with an age structure RP or Q are considered here. Beech forests are habitats of the protected black stork (*Ciconia nigra*), Ural owl (*Strix uralensis*), and many other species.

The component for **raised bogs, springs, fens and transitional mires** (Fig. 4) combines data from five biotopes (R2.2 Acidic moss-rich fens, R2.3 Transitional mires, R3.1 Open raised bogs, R3.2 Raised bogs with *Pinus mugo*, R3.3 Bog hollows) and covers 21 km^2. This area is also an important habitat of many endangered plants and invertebrates. Among many others also *Carabus menetriesi*, which is protected in the SCI Šumava, occurs there.

The last habitat component combines data on **other valuable Natura 2000 biotopes** and their mosaics and covers the area of 97 km^2 (Fig. 5). *Nardus* meadows and other secondary grasslands, many of them with blocked or very slow succession, are important biodiversity hotspots in the region, which is mostly covered by forests.

The fifth layer is showing the **core areas of capercaillie** (*Tetrao urogallus*) occurrence and covers 183 km^2. Five large and four smaller core areas (Fig. 6) were defined according to population monitoring held by NP Administration and Czech Ornithological Society, delineation was consulted with local experts as well.

The sixth layer (Fig. 7) shows **river canyons phenomenon** – the Křemelná River, the Otava River and



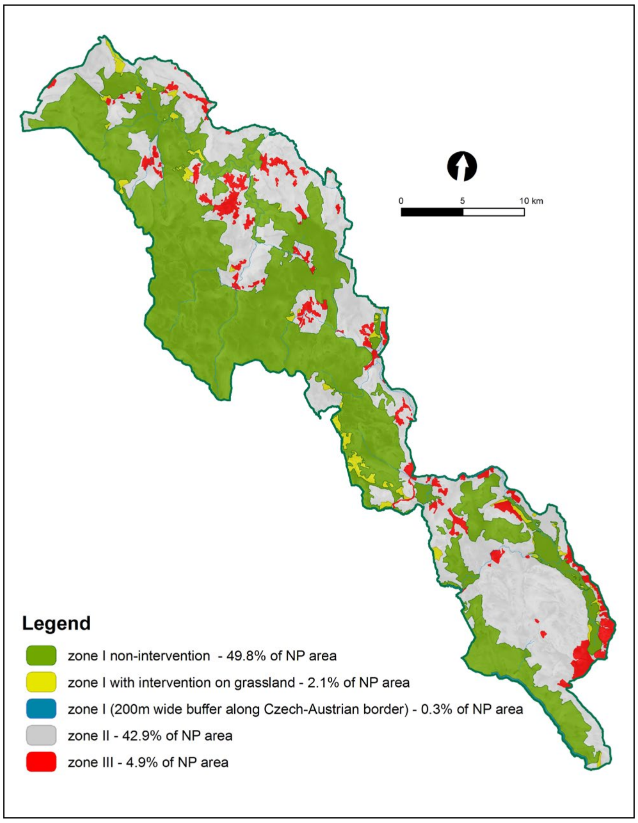


Fig. 8 The proposal of new zonation for the Šumava NP.

the Vydra River canyons. The important species like the lynx (*Lynx lynx*), otter (*Lutra lutra*) or peregrine (*Falco peregrine*), which are sensitive to human-caused disturbances, live in these canyons. Bullheads (*Cottus gobio*), a species protected in the SCI Šumava, is common there.

The final proposal is presented in Fig. 8 in which 49.8% of the area of the Šumava NP that is not managed and 2.1% of the permanently managed secondary grassland are included in Zone I. Zone III remains as it is (4.9%) and contains built-up and potentially built-up areas according to Act No. 183/2006 Coll. Planning and Building Regulations. Zone II includes the remaining 42.9%.

Discussion

The current zonation of the Šumava NP was criticized because the core zone (Zone I in Czech legislation) is extremely fragmented (135 units) and very small being only 13% of the area of the Šumava NP (Šolar and Galland 2002). Therefore, it is essential to establish a new system zonation. Several proposals were suggested recently. All of them define almost identical core zones. The recently published scientific proposal (Křenová and Hruška 2012) assumes that both the quality of the conditions and potential for natural development in the Šumava NP are high. They propose that Zone I includes 39.2% of the area of the Šumava NP (18,233 ha of forest plus 1,202 ha of grassland) and published biological arguments for including 15 main units in Zone I. However, this proposal, submitted to the Ministry of Environment, is the very minimum of what is required.

In this paper we present a more generous proposal of Zone I that should include 52.2% of the Šumava NP. The 15 units proposed by Křenová and Hruška (2012) are now included in larger units, mainly composed of spruce forests or beech forests generated by the Natura 2000 mapping data.

Since 1995, when the current Šumava NP zonation was implemented, most of the forest ecosystems were intensively managed and fragmented mainly due to the logging of trees attacked by bark-beetle. As a result, the stability of the forest in terms of the ability of the trees to withstand the damaging effects of high winds decreased and large areas of the Šumava NP forest were badly damaged by the windstorm Kyrill in January 2007. Natura 2000 habitats were used at that time to define the non-intervention areas (Křenová 2008), especially those habitats that thrive well without human intervention (montane spruce forests, peat bogs etc.). As a result, a non-intervention management policy was planned in 2007 in almost 30% of the Šumava NP area: in the whole of Zone I and certain parts of Zone II, especially in mountain spruce forests at high altitudes and water logged spruce forests.

Non-intervention management is not suitable for secondary habitats or small and fragmented areas. Therefore, this approach should focus mainly on primary habitats and large areas capable of self-restoration (Hußlein et al. 2009). Experience in other EU countries shows (EC 2012) that at large Natura 2000 sites the appropriate management is a combination of non-intervention management of primary habitats with permanent management of small patches of secondary habitat (e.g. secondary grasslands, juniper pastures). This is the approach used in the Bayerischer Forest NP (Kiener et al. 2008).

We show that data from the Natura 2000 mapping project can be used to define the optimum pattern of zonation for the Šumava NP while including other habitats like river canyons and the locations for particular species and taking into consideration the limitations imposed (e.g. by international agreements, buffer zones) and local knowledge of the area.

We believe that the Šumava NP and other large protected areas in Europe include excellent examples of particular natural habitats and can be used for determining the effects of natural disturbance and climate change. Long-term management strategies and good zonation are essential tools for this task.

Acknowledgements

The authors wish to thank to several local experts for their recommendations. We also thank very much prof. A.F.G. Dixon for revising the language and two referees for their valuable comments. This research was supported by the grant No. CZ.1.05/1.1.00/02.0073 of the MSMT.

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NEGOTIATING SUSTAINABLE INNOVATION? HYDROGEN AND FUEL CELL TECHNOLOGIES IN GERMANY

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ABSTRACT

Recently, the German Federal Government made the consequential decision to change its energy program. This not only as a result of the decision to shut down the existing nuclear power plants within the next few years, but also due to vital challenges like climate change and security of energy supply. The shift in the energy-technology paradigm from fossil fuel technologies to regenerative energies might appear as a merely technical process at first glance. Yet, the road to environmental sustainability is paved with economic and social stumbling blocks. The concept of sustainable development is not a blueprint for technical progress but requires deliberations on questions about innovations and governance: How do we want to live and how do we want to get there?
This paper traces the negotiations of sustainable innovation on the example of hydrogen and fuel cell technologies in Germany. The institutional set up in this field is analyzed and the new organizational actors are identified. These actors attempt to inform and persuade others of the benefits of hydrogen and fuel cells in order to establish a common view that is to guide the further development. However, while they succeeded in mobilizing enough actors to launch the largest Public Private Partnership in this sector in the EU, they could not attain the leadership in the public discourse on these technologies. It seems that an attractive guiding vision of a sustainable, post-fossil energy future and a broad acceptance in daily use would have been major prerequisites for such leadership.

Keywords: sustainability, hydrogen and fuel cell technologies, guiding vision, experience in use, visibility

Introduction

Since the Fukushima nuclear power plant disaster in March 2011, there has been a further increase in the already widespread dissent in Germany on energy policy. Simultaneously, the imminent threat of climate change is making a shift from fossil-fuel based energy sources to renewable ones inevitable. For many years, this dissent has blocked the emergence of a common EU energy policy (McGowan 1989; Pointvogl 2009), with the European Commission playing the role of a mediator among a group of heterogeneous member states with diverse national interests (Hancher 1994; Natorski and Herranz-Surrallés 2009). Each country's view on energy policy is shaped by the economic, technical and political structure of its national energy markets (Deàk 2009). Furthermore, different technical standards have prevented a consensus among the countries on energy policy (Geden and Fischer 2008). Even on the national level energy policy often is heavily contested. In Germany, the post-Fukushima decision to turn away from nuclear power will accelerate the shift to renewables. But the growing share of renewable energy is causing new conflicts as to whether new transmission lines should be built overhead or underground. There is also the question of how to organize and finance huge storage capacities in order to bridge the gaps in coverage caused by the stochastic character of sun and wind power. Thus the energy turnaround in Germany is highly challenging not only from a technical but also from a social and political perspective.

Hydrogen and fuel cell technologies are being re-examined in connection with this energy turnaround and the environmental sustainability. Germany is one of the leaders in this technology field, and hydrogen and fuel cells hold an exceptional position. New actors have emerged arguing for a comprehensive consensus on energy policy in the context of the expected paradigm shift. These actors can be labelled 'consensus agencies' as they have reached a sort of lowest common denominator regarding the promotion of alternative energy technologies while attempting to attract further actors to join their alliance. What prospects for success do these consensus agencies have in their bid to establish a comprehensive view on alternative energy technologies?

Background

Shift in the energy-technology paradigm

The contemporary energy system will be radically transformed in the 21st century, and the expected changes are often labelled as the 'new industrial revolution' (BMU 2008). At the core of such revolution is a shift in the energy-technology paradigm: away from fossil energy technologies to renewable ones. This paradigm shift is enforced through two pivotal global trends: Firstly, in the future there will not be enough cheap crude oil for worldwide economic growth (Schindler and Held 2009). Secondly, greenhouse gas emissions will lead to considerable changes in global climate, and the growing awareness of climate change has strengthened environmental policies and supported the development of renewable energy technologies (Christiansen 2002; Stern 2006).

Recently, the International Energy Agency claimed the "end of cheap oil" and predicted a supply shortfall (IEA 2010). Both the decreasing oil production and the growing oil demand will lead to a rise in oil prices. Hence, economic growth (the gross domestic product – GDP) needs to be decoupled from oil – and alternative, non-fossil energy technologies must be developed.

The other process that promotes this development is climate change. A rise in global surface temperature has been observed since 1850, when instrumental recording began (IPCC 2007). The concentration of greenhouse gases in the atmosphere has increased since 1750 as a result of human activities, in particular with the beginning of industrialization (IPCC 2007). Though it was long contested whether these two processes are related, it is very likely that global warming is caused by humans. Both the concentration of greenhouse gases and the resulting rise in temperature have been characterized by exponential growth since the beginning of the 20th century (IPCC 2007). This growth will lead to considerable changes in global climate (Luhmann 2008; Bahn et al. 2011).

The challenge though is not only a transformation from fossil to non-fossil sources but to those renewable energy sources whose production and usage allows a CO_2-free energy cycle. Hydrogen and fuel cells have the potential to fulfil these requirements (Praetorius 2009).

Hydrogen and fuel cell technology

Hydrogen and fuel cell technologies are among the most promising new energy technologies. Their linkage opens up the chance to deploy renewable energy sources in transportation, electricity and heat generation in CO_2-free energy cycles. They target an area which is currently responsible for half of the EU's total greenhouse gas emissions (Van Vliet et al. 2011). For two reasons, however, this may not be an easy task.

Firstly, the term "hydrogen and fuel cell technology" suggests a combination of two technologies which is possible, but not mandatory. Hydrogen can be used without fuel cells, for instance as fuel for internal combustion engines in vehicles. Likewise, fuel cells can be powered by fuels other than hydrogen, such as methanol. Furthermore, there is a substantial difference between the two technologies: hydrogen is an energy carrier while fuel cells are energy converters. That means in effect, hydrogen and fuel cells are the combination of an energy carrier and an energy converter technology. This combination is a broad application area of both technologies, but not the only one.

Secondly, it should be noted that both technologies are not ecological per se. As hydrogen rarely exists in a pure gaseous form in nature, it has to be obtained from hydrogenous compositions. Here, a variety of possible production processes comes in. Hydrogen can be generated from coal, natural gas, biomass and water. Each production process results in a different energy cycle. Fuel cells can be powered by methanol and hydrogen, which can be produced from different raw materials and in a variety of ways, so that both may result in completely different energy cycles.

Mostly, the supporters of hydrogen and fuel cell technologies do not promote these in general, but with regard to their ecological potential. They envisage "green" hydrogen and fuel cell technologies that rely on renewable energies and contribute to a CO_2-free energy cycle – instead of black or brown technologies that are based on fossil energy sources. Therefore, to speak of a CO_2-free energy cycle, the entire fuel process chain has to be considered. This concerns the fuel pathway from "fuel processing from the primary energy source" to its use "by the propulsion technology that converts fuel to motion on board the vehicle" (Ramesohl and Merten 2006).

In the case of hydrogen, only hydrogen production from renewable energies can contribute to a CO_2-free energy cycle (Ramesohl and Merten 2006). Hydrogen and fuel cells can be used to generate power and electricity as well as to run small-scale heating devices for private households and large-scale devices for industry. They can provide power for small, portable applications such as mobile phones and notebooks, but can also serve as a propulsion system in large vehicles.

In Germany, due to the great economic importance of the automotive industry, most attention is actually paid to transport applications. The transformation of the current CO_2-emitting energy system into a CO_2-free one that is based on hydrogen and fuel cell technologies is strongly associated with a sustainable transport system. The guiding vision would be the image of hydrogen that is produced from renewable energy sources and then used as transport fuel to power fuel cell driven vehicles. In this way, hydrogen and fuel cell technologies could enable a CO_2-free energy cycle from its generation to end-use.

Policy context

Alternative energy technologies at an interface

The primary objectives of official German energy policy are economic efficiency, security of energy supply, and environmental compatibility (BMWi 2010). Since 1974 the Government has continuously developed Energy Research Programmes, conducted under the auspices of different Federal Ministries (from "Economics and Technology" to "Environment, Nature Conservation and Nuclear Safety", "Consumer Production, Food and Agriculture", and the "Ministry of Education and Research"). This illustrates that alternative energy technologies are embedded in a broad policy context.

For instance, batteries, fuel cells and bio fuels have in common that they can become components of propulsion systems in the transport sector. In this way, they do not only attract environmental interests, as they can contribute to reduce CO_2-emissions, but also economic interests. To remain competitive, the German automotive industry has to keep track of the newest developments in propulsion systems. Furthermore, energy efficient technologies are of great importance for the entire economy. And they are needed to reach the climate policy targets that Germany has agreed to in international contracts.

Hydrogen and fuel cell technologies have been part of several research programmes for many years. Their funding increased continuously from 1974 and reached a provisional peak in 1994 (BMVBS et al. 2006). The most important time period in hydrogen and fuel cell funding however was from 2006 onwards, when hydrogen and fuel cell technologies gained their exceptional position. This development was coined by the National Hydrogen and Fuel Cell Technology Innovation Programme (BMVBS et al. 2006) and the National Development Plan in 2006 and 2007 (SCHFC 2007). The Federal Government (represented by the Ministry of Transport, Building and Urban Affairs) launched the National Organization of Hydrogen and Fuel Cell Technology (hereinafter NOW) in 2008. This organisation consists of a management, a supervisory board and an advisory board, composed of members from industry, politics and science (NOW 2010a; NOW 2010b). Its task is to prepare the market entrance of hydrogen and fuel cells by coordinating and financing demonstration projects.

The financial support increased rapidly. The budget of NOW amounts to € 1.4 billion up to 2016. Hence, hydrogen and fuel cell technologies will be funded by at least € 100 million per year from 2008 to 2016. This annual funding exceeds the average annual funding of 1974–2004 by a factor of ten or more (BMVBS et al. 2006).

Negotiating sustainable innovation

The role of consensus building agencies

The strong position of the hydrogen and fuel cell community in Germany relies on beliefs and expectations of diverse actors on a promising technological future. These shared beliefs and expectations were not given by nature but rather result from the work of consensus building agencies. Consensus building agencies are amalgamations of diverse actors from economy, science and politics that agreed upon a certain view on hydrogen and fuel cell technologies and that attempt convincing further actors of sharing this view.

In this subchapter we will illustrate how the work of three consensus building agencies brought hydrogen and fuel cell technologies into the exceptional position that they are holding in German energy policy. The three consensus building agencies have been very successful in setting and keeping hydrogen and fuel cell technologies on the agenda and in mobilizing diverse actors by aligning their interests. However, while there finally is a broad consensus on hydrogen and fuel cell technologies shared by many diverse actors, the actual realization of a sustainable transport remains far away as will be outlined in subchapter 4.2.

The consensus building process in Germany was characterized by a bottom-up approach, primarily influenced by the three consensus building agencies: The Transport Energy Strategy (hereinafter TES), the Clean Energy Partnership (hereinafter CEP) and the National Organization Hydrogen and Fuel Cell Technology (hereinafter NOW) For a long time, hydrogen and fuel cell technologies had been promoted on a low political level along with other alternative energy technologies. It was the TES that raised them to the exceptional position that they hold at present.

The TES is an amalgamation of energy companies and car manufacturers that agreed upon the conclusion that hydrogen is the most promising fuel for the future transport sector. The initially thin consensus was continuously broadened; internally and externally. The loose idea of hydrogen as the fuel of the future was concretized, and certain steps were agreed upon towards the realization of the idea. This consensus attracted further actors who joined the movement. The TES initiated the Clean Energy Partnership (hereinafter CEP) which is the largest hydrogen and fuel cell technology demonstration project in the EU, and a concrete step towards the practical implementation of the consensus. Furthermore, the TES lobbied this issue upwards in politics. The following paragraphs describe the importance of the consensus building agencies in setting hydrogen and fuel cell technologies on the agenda and in aligning the interests of diverse actors (for more details see Marz 2010; Marz and Krstacic-Galic 2010):

Transport Energy Strategy (TES): The TES was launched in May 1998 by the Federal Government, represented by the Ministry of Transport, Building and Urban Affairs, and the private enterprises ARAL, BMW, Daimler, MAN, RWE, Shell and Volkswagen. Later on Ford, GM/Opel, Total and Vattenfall joined it. The objective of the TES was to develop a strategy that should secure an international leading position for Germany in the field of alternative energies and their production and application in the transport sector over the next 10 years. Further objectives were to reduce the sector's dependency on oil as well as to reduce emissions, in particular CO_2-emissions, and to extend the TES onto the European level.

The daily work is done by a Steering Committee that receives the reports of the Task Force and provides recommendations to the organizations involved. In addition, the TES instructs third parties such as the Ludwig Bölkow Systemtechnik, which is a highly respected consulting company in the field of hydrogen and fuel cell technologies worldwide. On the basis of the results the TES established an initial internal consensus. Thus the

TES is not a consensus agency that supported the development of hydrogen and fuel cell technology from the beginning but rather became one due to an internal consensus building process. In recent years it has tried to specify the common objective in more detail: Hydrogen should be produced from wind power, photovoltaic, hydro power, solar and geothermal energy.

Furthermore, the TES defined which actions had to be taken to reach their goal and decided to initiate national and European demonstration projects to illustrate the suitability for daily use of hydrogen and fuel cell technologies. Among others, it launched CEP, the largest demonstration project in the EU. The TES also lobbied towards a common European platform for the promotion of hydrogen and fuel cell technologies and succeeded, as the launch of the Fuel Cell and Hydrogen Joint Undertaking (FCHJU) by the Council of the European Union indicates. Finally, the TES suggested the development of the NIP, and also succeeded.

Clean Energy Partnership (CEP): The CEP was set up in October 2003. It is composed of the car manufacturers BMW, Daimler, Ford, GM/Opel and Volkswagen, the energy supplying companies Aral, Linde, StatoilHydro, Total and Vattenfall, and the transport companies BVG and Hamburger Hochbahn. Some of these actors are also involved in the FCH JU, which allows them to promote the consensus of the CEP internationally. Furthermore, the Federal Government is involved in the CEP, represented by the Ministry of Transport, Building and Urban Affairs. It funds the project with up to € 5 million in order to support the construction of a hydrogen infrastructure.

The consensus upon which the CEP rests is very detailed and long-term. The shared ambition of the actors is to work towards a 'silent and clean transport system'. Hydrogen is thought to become the energy carrier of the future, when produced from renewable energies, thus enabling their deployment in the transport sector. In this way, "green" hydrogen shall function as the fuel for all types of vehicles and thus produce pure water as a by-product instead of harmful emissions. The actors intend to construct hydrogen filling stations and to test hydrogen powered vehicles in order to develop the complete energy cycle from hydrogen production to its usage.

National Organization Hydrogen and Fuel Cell Technology (NOW): Before the launch of the NOW, several consensus agencies co-existed side by side. In 2001, actors from industry and science brought BERTA into being, a working group with the task of positioning the fuel cell technology in the investment programme of the Federal Government. In 2003, the Ministries of Economics and Labour, of Transport, Building and Urban Affairs, and of Environment, Nature Conservation and Nuclear Safety launched the Hydrogen Strategy Council (HSC). This council comprised experts from industry, science and representatives of Federal and State Ministries in order to coordinate the national activities in the field of hydrogen. As hydrogen and fuel cell technologies overlap and complement each other, all involved interests were merged in a new amalgamation called HyBERT in February 2005. HyBERT was an advisory council with the task to advise ministries, to define Research and Development requirements and to serve as a platform for the exchange of information. Later on in 2005 HyBERT was renamed into the Strategy Council H2 and Fuel Cells.

The main task of the NOW is to coordinate the demonstration projects in order to push hydrogen and fuel cell technologies towards market entry. The NOW funds more than 35 such demonstration projects; the most important of these is the CEP. From 2008–2011, the NOW provided 48 percent of the total budget of € 25.8 million of the CEP (see Fig. 1 for details).

The way in which the consensus agencies frame these technologies allows various actors to identify a certain position for themselves in a future energy system that is based on hydrogen and fuel cells. Although heating service companies and car manufacturers have little in common, both can identify with the overall consensus and share an interest in the development of hydrogen and fuel cell technologies. They participate in the NOW as the consensus is broad enough to capture diverse interests. But the specific task of the NOW is to implement the shared agenda by demonstration projects. The architecture of the "consensus landscape" in Germany is illustrated in Fig. 1.

Obstacles to a sustainable transport system based on hydrogen and fuel cell technologies

Despite the success in reaching a consensus, there are still apparent stumbling blocks on the road to a sustainable transport system relying on fuel cell vehicles powered by hydrogen generated through renewable energies. The two most salient issues are to be elaborated further in the following paragraphs: hydrogen production and end-user involvement.

The official long-term objective of the consensus building agencies mentioned above it to produce hydrogen from renewable energies in order to establish an emission-free cycle from hydrogen production to vehicle propulsion. Thus reference to the ecological dimension of the development and use of the "green" hydrogen and fuel-cell technology is made in almost all of the programmes and projects initiated. In NOW's guidelines for the evaluation of lighthouse projects, for example, the ecological focus constitutes an integral part of each project. In particular, the projects "should take into account the aims of the Federal Government with regard to increasing energy efficiency, the conservation of resources and climate protection" (NOW 2010c). Furthermore, the lighthouse projects should adhere to the Kyoto Protocol, the government's climate protection obligations and its climate protection programme, as well as the energy saving regulations, the building renovation programme and

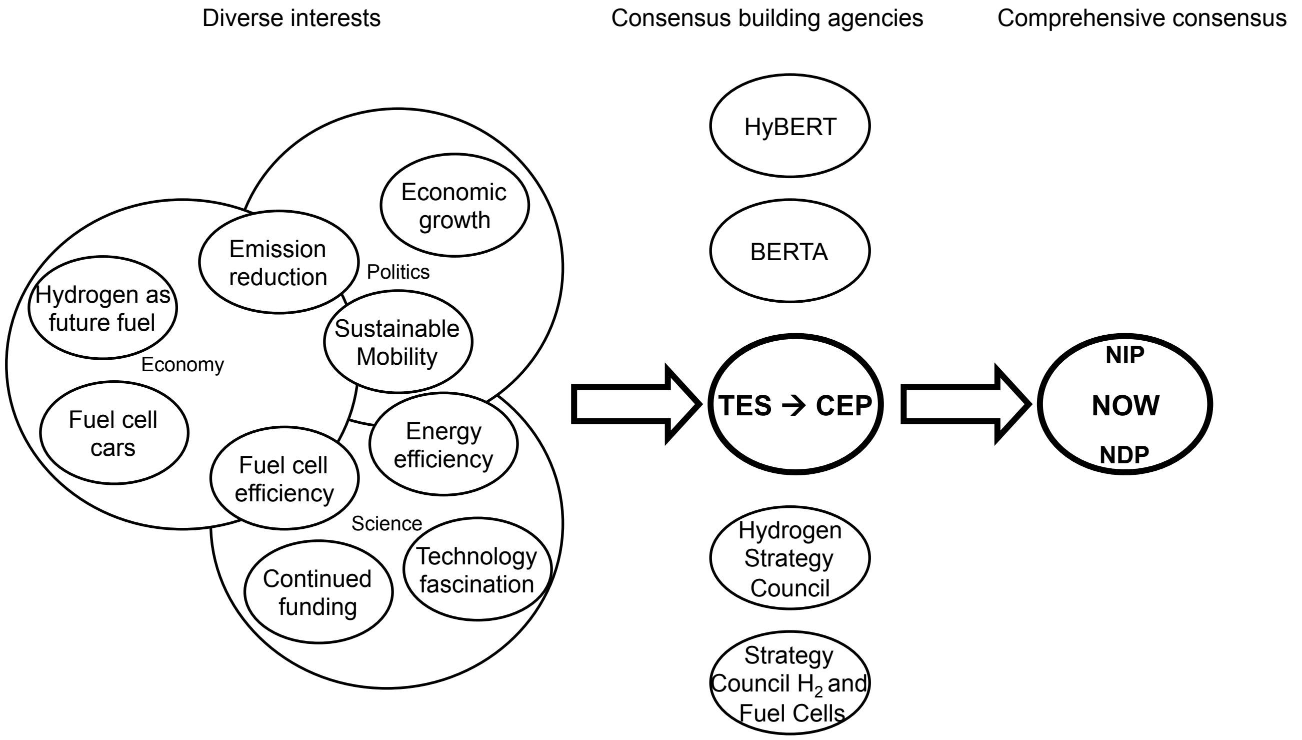

Fig. 1 The Consensus Building Process in Germany. Abbreviations: BERTA: Working Group for fuel cell technologies, HyBERT: Advisory Coouncil for hydrogen and fuel cell technologies.

the aims of the Combined Heat and Power Act (Kraftwärmekopplung – KWK), as well as the renewable energy legislation (NOW 2010c).

However, a look at the reality today clarifies how far these long-term objectives are from being achieved. In fact, more than 90 percent of the hydrogen currently produced in Germany and classified as an industrial by-product is generated from fossil sources. Therefore, critics of hydrogen and fuel-cell technologies doubt that these technologies will ever meet the environmental expectations placed in them. They call attention to the unsolved problems in connection with the transformation/conversion losses and consider them to be technical and economic aberrations, rather than "green" technologies for the future (Bossel 2006; Heise Autos 2007).

Apart from the problems of ecological impacts, further obstacles to the realization of a sustainable transport system can be identified in the way hydrogen and fuel cell technologies are being developed. Engineers and infrastructure designers have strongly influenced previous projects. These actors are often situated far from the consumer or end-user markets; their interests lie in the functional aspects of new technologies, their safety, reliability, and compatibility.

Yet, new products are no longer bought and used only on a functional basis but also, or perhaps even because of their cultural aspects. Not only do they influence lifestyles, they also send out signals, indicating the social status of the owner of products, such as notebooks, mobile phones or automobiles. Certain products are highly attractive and have reached an unexpectedly high level of dissemination. They satisfy the already existent but also the latent needs of the user. They can be easily integrated into and become part of the user's everyday life. Finally, they have a clear gain of distinction for the owner or user. The willingness of the customer to pay for these products is high. These products have a much greater symbolic value than a functional or instrumental one. In sociological terms, the possession of, or access to these products is the visible proof of social inclusion.

The findings described above show that functional advantages alone do not play the decisive role in commercial success. Rather, a number of social and cultural qualities are essential. Hydrogen and fuel cell technologies have to appeal to the consumer if a mass market is to be established and environmental impacts are to be achieved. For this purpose, they may not only be considered as background technologies that have to fulfil a practical function but they also have to be considered as a kind of status symbol. Concrete experience with new product characteristics is of importance for consumers. One such specific experience is the low noise level of a fuel-cell drive engine.

Prospects of the consensus

As already illustrated above, a crucial point for a sustainable transport system based on hydrogen and fuel cell technologies is that hydrogen production is only sustainable and emission free when it relies on renewable energies. Studies conducted on behalf of the TES and the Federal Ministry of Transport, Building and Urban Affairs indicate that hydrogen could be largely produced from renewable energies in 2020 (TES 2007), or 2050, respectively (BMVBS 2009). But unless the actual share of renewable energies in electricity production does not increase considerably, sustainability of hydrogen and fuel cell technologies will remain a contested issue.

A further impediment in the process of reaching a consensus is the tenuous relationship that these technologies have with electromobility, a technology which, since 2008, has been experiencing an unexpected revival in policy innovativeness (Canzler and Knie 2011). Although there is competition between both technologies, they also complement each other. Hydrogen and fuel-cell driven automobiles have a larger operating range than battery-driven vehicles. They can supplement each other inasmuch as hydrogen and fuel-cell drives are suited for long-distance transportation, while battery-driven vehicles are predominantly practical for city and local traffic (BMVBS 2011). Since fuel-cell and battery vehicles both use electric drives, there is potential competition with regard to which one has the 'better' electric drive. Should the complementary relationship assert itself, it would result in a win-win situation for both technologies. Should the competition relationship succeed, it could easily result in a lose-lose situation.

Conclusion

Alternative energy technologies provide new opportunities for a sustainable consensus in energy policy. These opportunities stem from the actions of various actors and, above all, new types of actors. Agencies whose main objective is to lobby towards a consensus on certain technologies can considerably influence the political agenda. They can frame the discussion in a way that allows them to gather diverse interests under one roof. This makes agreements possible which may pave the way for more sustainable forms of consensus.

Our case study of the hydrogen and fuel cell community in Germany has shown the opportunities, the fragility and the prospects of such agreements. While new technologies can lead to new interest constellations and overcome dissents, they can likewise create new lines of conflict. Although a general consensus on hydrogen and fuel cell technologies could be achieved, it could dissolve again soon if the critique concerning the sustainability of hydrogen production is not adequately tackled. The share of renewable energies in hydrogen production has to increase if hydrogen and fuel cell technologies are to contribute to a sustainable transport system.

The other main obstacle towards a sustainable transport system based on hydrogen and fuel cell technologies is the insufficient involvement of the end-users in the development.

In modern societies, sustainability is closely linked with a plausible, transparent and reflexive way of dealing with the opportunities and risks of innovations. Although there is a great deal of uncertainty involved, an attitude of openness towards the possible effects of a new technology is needed. Also, the need for social participation cannot be ignored. Thus the end-users have to be included in the development of hydrogen and fuel cells; otherwise these technologies might not reach a broad societal acceptance and usage.

Eventually, hydrogen and fuel-cells could play a significant role as clean energy technologies in the future post-fossil age. Yet, the road towards a sustainable transport system is not a smooth one. The transformation of the energy basis will be accompanied by discontent and irritation due to the devaluation of traditional energy technologies and their know-how carriers. Furthermore, new storage and transmission capacities will be needed, meaning that natural space will be affected. An increase in decentralized energy production may disrupt landscape aesthetics.

Concepts such as "smart grid" or "100 percent renewable energies" are highly demanding (Schindler and Held 2009). Whether or not hydrogen and fuel-cell technologies will acquire social acceptance depends not only on their technical efficiency and the reduction of operating costs, but also on the broad implementation of basic technologies to produce attractive products. An attractive product is one that fulfils the consumer's existing (or latent) needs, is easy to operate, has a stylish design, is suitable for everyday use, and at the same time enables the user to accentuate social distinction. A decisive factor therefore will be if and when hydrogen and fuel-cell technology and their associated products can become integrated into daily routines.

These findings on the German case are in line with international investigations. Sovacool and Brossmann have illustrated how hydrogen attracts various interests due to promising expectations (Sovacool and Brossmann 2010); Bakker highlights the fragility of the consensus if these are not fulfilled (Bakker 2010). Therefore, the protagonists of new technologies must be able to provide a plausible answer to the question whether the new technology will be sustainable in the long term, both for humankind and the natural environment. The days of blind faith in technology are over, and new technologies are no longer automatically regarded as superior to existing ones.

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