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Use of oleaginous plants in phytotreatment of grey water and yellow water from source separation of sewage

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ABSTRACT

Efficient and economic reuse of waste is one of the pillars of modern environmental engineering. In the field of domestic sewage management, source separation of yellow (urine), brown (faecal matter) and grey waters aims to recover the organic substances concentrated in brown water, the nutrients (nitrogen and phosphorous) in the urine and to ensure an easier treatment and recycling of grey waters. With the objective of emphasizing the potential of recovery of resources from sewage management, a lab-scale research study was carried out at the University of Padova in order to evaluate the performances of oleaginous plants (suitable for biodiesel production) in the phytotreatment of source separated yellow and grey waters. The plant species used were *Brassica napus* (rapeseed), *Glycine max* (soybean) and *Helianthus annuus* (sunflower). Phytotreatment tests were carried out using 20 L pots. Different testing runs were performed at an increasing nitrogen concentration in the feedstock. The results proved that oleaginous species can conveniently be used for the phytotreatment of grey and yellow waters from source separation of domestic sewage, displaying high removal efficiencies of nutrients and organic substances (nitrogen > 80%; phosphorous >90%; COD nearly 90%). No inhibition was registered in the growth of plants irrigated with different mixtures of yellow and grey waters, where the characteristics of the two streams were reciprocally and beneficially integrated.

Introduction

The traditional concept of using huge quantities of water to transport domestic waste away from households, resulting in the production of diluted wastewater streams and treatment at centralized facilities, has often been reconsidered due to the related costs, high use of resources and significant surface occupancy (Butler and Parkinson, 1997; GTZ, 2003; Gandini, 2004).

More and more attention is being focused on sustainable sanitation systems, aimed at closing nutrient and water cycles, with low material and energy consumption. In these systems, sewage is considered a valuable source of nutrients and water for plant growth. Sustainable sanitation systems are generally based on collection and treatment of different source-separated sewage streams: yellow water (urine); brown water (faeces) and grey waters from kitchen, laundry, dishwasher, shower, *etc.* (Langergraber and Muellegger, 2005; Cossu et al., 2003a, 2003b;

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Borin et al., 2004). Source separation is carried out to optimize the potential for reuse when compared to "end-of-pipe" technologies (Larsen and Maurer, 2011).

Depending on the purpose of reuse, several studies focusing on the treatment of source-separated sewage streams applied technologies largely similar to those adopted in the conventional treatment of combined wastewater (Jefferson et al., 1999; Maurer et al., 2006; Escher et al., 2006; Kujawa-Roeleveld and Zeeman, 2006; Leal et al., 2010; Larsen and Maurer, 2011; Saeed et al., 2014; Zhang et al., 2015), whilst only a few cases have been studied and used for the phytotreatment of grey waters (Frazer-Williams et al., 2008; Fangyue et al., 2009; Vymazal, 2009).

A sustainable source-separated system, named "Aquanova", has been developed since the early nineties at the University of Padova. The system is aimed at optimizing the integrated management of various source separated sewage streams and biodegradable fractions of solid waste (Cossu et al., 2003a, 2003b).

The Aquanova system is graphically described in Fig. 1. Three different sewage streams are segregated using a source separation toilet and separate piping for grey water outflows. Yellow water and grey waters undergo phytotreatment, while brown waters mixed with shredded kitchen waste undergo anaerobic digestion.

Several aquatic plant species – such as *Acorus calamus Variegatus*, *Alisma plantago aquatica*, *Calla palustris*, *Canna indica*, *Eupatorium cannabinum*, *Iris pseudacorus*, *Lythrum salicaria*, *Lobelia cardinalis*, *Lysimachia nummularia*, *Mentha aquatica Rubra*, *Thalia dealbata*, *Typha latifolia*, *Lemna minor*, *Eichornia crassipes*, *Phragmites australis*, *Typha* – and natural mountain flora – such as *Aconitum napellus*, *Senecio cordatum*, *Senecio rupestre*, *Epilobium alpestre*, *Achillea millefolium* – have been tested in lab-scale and full scale phytotreatment units under different operative conditions, in previous research programmes performed by the authors of this paper (Cossu et al., 2003a).

The results of these studies confirmed the good performances of a wide species of plants in the phytotreatment of grey and yellow waters (Borin et al., 2004).

Considering the interest developed in recent years in the production of alternative energy from oleaginous crops, and the related concern for competing land use by energy crops (the "table or tank dilemma"), the present research was conceived in order to investigate the phytotreatment of source segregated sewage fractions using oleaginous crops active under temperate climatic conditions such as soybean (*Glycine max*), rapeseed (*Brassica napus*) and sunflower (*Helianthus annuus*), already taken into consideration for use in the production of industrial biodiesel (Lavagnolo et al., 2016, Meher et al., 2006; Zegada-Lizarazu and Monti, 2011). In particular, biofuel obtained from sunflower and rapeseed was found to be of excellent quality due to the high content of monounsaturated esters (Ramos et al., 2009).

1. Materials and methods

1.1. *Wastewaters*

The experiment was carried out at the Environmental Engineering Centre, Department of Industrial Engineering, University of Padova, where the Aquanova system has been implemented.

The following waters were used as feedstock: grey waters from bathroom sinks (GW); kitchen waters from kitchen sink (KW); yellow waters (YW) from a source segregation toilet (Fig. 2a).

Wastewaters samples were analysed according to the Italian standard analytical methods (CNR-IRSA, 29/2003) and measured in triplicate. pH, alkalinity, total solids (TS), volatile solids (VS), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), N-NH_4^+ and the other parameters listed in Table 1 were taken into account to characterize the feedstock. COD was evaluated by the potassium dichromate oxidation method. BOD_5 was evaluated using a respirometer apparatus (Sapromat E). BOD_5 of kitchen water was

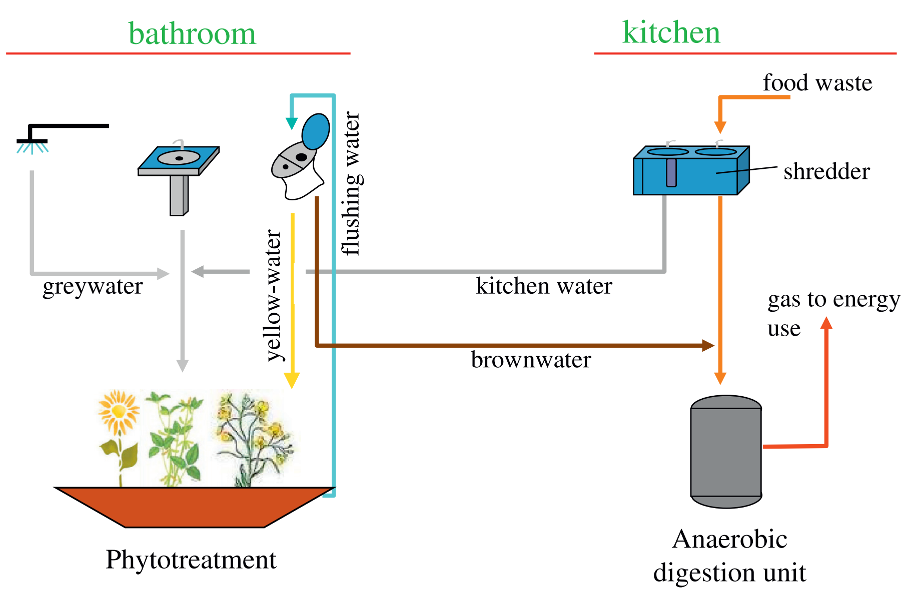

Fig. 1 – Scheme of the Aquanova system for the integrated management of sewage and kitchen waste (Cossu et al., 2003a).

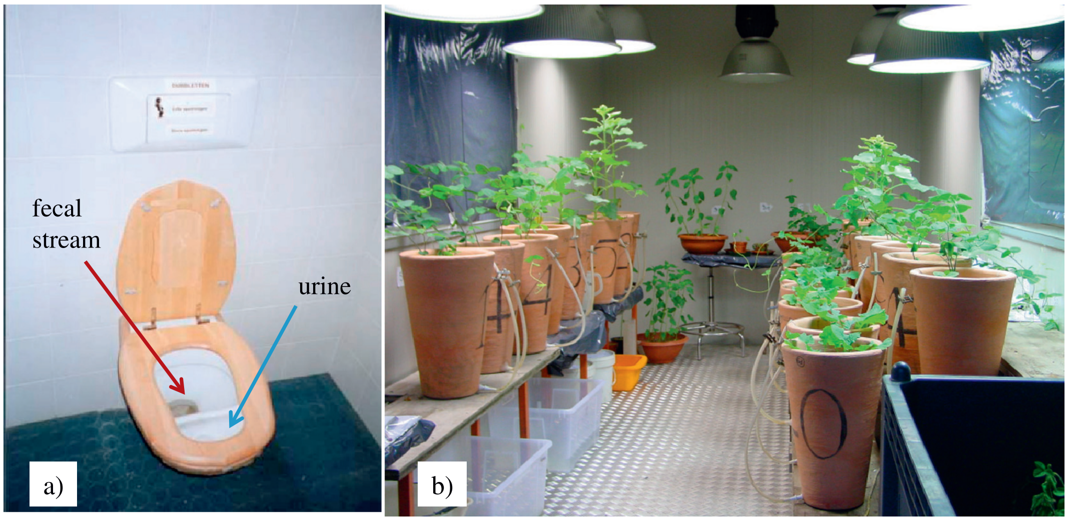

Fig. 2 – Equipment used in the research: (a) source separate toilet; (b) greenhouse.

performed after pre-filtration at 2 μm in order to detect the soluble BOD compounds. TKN and N-NH_4^+ was evaluated by means of a distillation-titration procedure, while TKN was measured after an acid digestion phase. Dissolved components (nitrate, phosphate and sulphate ions) were determined using a UV–VIS spectrophotometer (UV-1601, Shimadzu, Japan) preceded by filtration with a 0.45 μm pore membrane. The colorimetric method was used to detect total phosphorus after sample digestion. Chloride and sulphide were measured by titration, whereas metal content was measured by Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES-4200 DV, Perkin Elmer, USA).

Wastewaters were analysed twice a week throughout the entire study period. The analytical results are summarized, as mean values, in Table 1. Heavy metals concentrations, with the exception of Cu and Fe, were below detection limits.

Table 1 – Mean values of pH and concentration of different analytical parameters monitored in the grey, kitchen and yellow water samples used in the phytotreatment runs.

	Grey water	Kitchen water	Yellow water
pH	7.7 ± 0.2	6.9 ± 1.5	8.0 ± 1.0
Alkalinity (mg $CaCO_3$/L)	258 ± 20	414.7 ± 37	5000 ± 925
BOD_5 (mg O_2/L)	30 ± 5	90 ± 5	842 ± 17
COD (mg O_2/L)	54 ± 17	1002 ± 80	2924 ± 76
TKN (mg N/L)	1.5 ± 0.4	0.8 ± 1.2	3320 ± 740
N-NH_4^+ (mg N/L)	1.5 ± 0.4	0.8 ± 1.2	3100 ± 504
P (mg P/L)	3.1 ± 0.1	5.3 ± 1.0	350 ± 130
TS (mg/L)	401 ± 10	987 ± 63	9387 ± 928
VS (mg/L)	133 ± 6	859 ± 130	3647 ± 999
Cl^- (mg Cl/L)	27.6 ± 1.1	29 ± 15	1597 ± 67
SO_4^{2-} (mg S/L)	23.9 ± 3.3	12.6 ± 4.2	187 ± 6.0
MBAS (mg/L)	0.30 ± 0.02	115 ± 52	–
Cu (μg/L)	72.2 ± 15	154 ± 110	117 ± 43
Fe (μg/L)	381 ± 59	239 ± 54	419 ± 77

BOD_5: biochemical oxygen demand, measured in 5 days; COD: chemical oxygen demand; TKN: total Kjeldahl nitrogen; TS: total solids; VS: volatile solids; MBAS: Methylene Blue Active Substances.

1.2. Plants and inflow waters

Phytotreatment tests were carried out in 20 L plastic pots with a layer of 30 cm of sandy substrate (sand: 82%, clay: 10%, silt: 8%; density: 1.5 kg/L) and a layer of 10 cm medium-sized gravel as bottom drainage. A drainage tube was fitted at the base of each pot to drain off the outflow. The analytical quality of the sandy substrate used is described in Table 2.

Three plant species were tested: *Brassica napus* (rapeseed), *Glycine max* (soybean) and *Helianthus annuus* (sunflower). Seeds were provided by the Seed Data Bank of the DAFNAE Department, University of Padova. Eight pots per each plant species were used: four as testing units and four as control units. One plant was grown in each experimental unit. The pots were arranged in a greenhouse (Fig. 2b) where an average daily temperature of 24°C, average night temperature of 12°C and a photoperiod of 14 hr were maintained.

At the beginning of the experiment (acclimatization period), all experimental units were irrigated using tap water and Hoagland's nutrient solution (Hoagland and Arnon, 1950) in order to promote initial plant growth. From the acclimatization phase onwards, testing units (four of each species) were watered first with grey water and subsequently with

Table 2 – Quality of the sandy substrate used in the experimental pots. Data are refer to dry solid matrix of the substrate.

Parameter	Value	Parameter	Value
TS (% w/w)	98.0 ± 1	Ca (mg/kg)	157,761.0 ± 156.0
VS (%TS)	1.2 ± 0.3	Cd (mg/kg)	< 0.7
TOC (%)	< 1.0	Cr (mg/kg)	2.5 ± 0.2
TKN (mg/kg)	77.6 ± 2.2	Cu (mg/kg)	6.5 ± 0.3
NH_4^+-N (mg/kg)	55.1 ± 1.8	Fe (mg/kg)	3955.8 ± 178.8
NO_3-N (mg/kg)	< 10.0	K (mg/kg)	810.2 ± 25.6
P-tot (mg/kg)	173.0 ± 12.2	Mg (mg/kg)	51,665.1 ± 223.2
Cl^- (mg/kg)	2278.9 ± 125.1	Mn (mg/kg)	179.3 ± 3.1
Si (mg/kg)	125.1 ± 8.3	S (mg/kg)	108.8 ± 2.7
Na (mg/kg)	357.5 ± 12.5	Pb (mg/kg)	2.7 ± 0.4
Ni (mg/kg)	3.3 ± 0.3	Zn (mg/kg)	20.7 ± 1.9

Table 3 – Description of the feeding mixtures adopted throughout the different research phases.

Phase	Duration (days)	Composition of the inflow water (%, V/V)
Acclimatization	19	tap water + nutritive solution (Hoagland solution)
	10	50% tap water +50% GW
Phase I	18	100% GW
Phase II	10	49.95% GW + 49.95% KW + 0.1% YW
Phase III	10	49.9% GW + 49.9% KW + 0.2% YW
Phase IV	10	49.75% GW + 49.75% KW + 0.5% YW

GW = grey water, KW = kitchen water, YW = yellow water.

different combinations of grey, kitchen and yellow waters. The research was divided into four phases, each characterized by different dosing, with the aim of gradually increasing the load of nitrogen. The duration and settings of the four phases are described in Table 3.

The remaining pots (four of each species) were watered with tap water and Hoagland's solution and used as control units according to a well established procedure (Holmes, 1980; Hocking and Steer, 1983; Salvagiotti et al., 2008).

Hydraulic loading was provided according to the individual plant growth demand and the hydraulic retention time (HRT) was kept equal to 7 days minimum in all experimental units. In a similar experiment, Sawaittayothin and Polprasert (2007) demonstrated that the minimum HRT must be between 5 and 8 days, depending on the contaminants to be removed. The experiment was extended for the entire vegetative period of the three species until plant senescence was reached (end of phase IV).

During the research phases the outflow streams from the different pots were sampled and analysed twice a week, according to the Italian standard analytical methods (CNR-IRSA, 29/2003), with four replicates.

At the end of phase IV the plants were individually uprooted. Shoot and root length and fresh weight were measured. The plant tissues were dried at 65°C for 3 days. Dry weight was measured and the content of total nitrogen (as sum of TKN and $N\text{-}NO_3$), total phosphorous, heavy metals and microelements was determined.

The sandy substrate was analysed for determining total nitrogen and total phosphorous contents, in order to evaluate the role of soil in nutrients removal. Plants and sandy substrate were analysed according to the Italian standard analytical methods for solid samples (CNR-IRSA, 64/1985).

2. Results and discussion

2.1. Wastewaters and feeding mixture quality

The quality of the wastewaters used in the research (Table 1) of course reflects the water use and residential peculiarities of the community where they were produced, the University (*e.g.*, no shower was utilized). For all parameters the observed values were, as expected, higher for yellow water rather than for grey water, but nitrogen content found as mean value in the last was lower than values found in other studies (Cossu et al., 2003b; Erikkson et al., 2009; Fangyue et al., 2009; Kattel et al., 2011).

Table 4 shows the quality of wastewater feedings measured during the different research phases. The concentration of nutrients (N and P) increased progressively, as purposely planned, from phases I to IV.

Nitrogen load was mainly associated to yellow water. This is clearly evident from the first phase feedings when only grey water was present, and consequently the nitrogen load in the inflow was particularly low. The yellow water acted as fertilizer for the plants, without overloading the hydraulic volume of the system.

COD and solids concentrations in the feeding were mainly linked to the presence of kitchen water and yellow water, while MBAS (Methylene Blue Active Substances) were mainly associated with kitchen waters.

Water demand naturally increased during the experimental period (Fig. 3), due to plant growth. Water demand reached a peak in phase III, whereas the reduction in water consumption during phase IV clearly underlined the onset of plant senescence.

2.2. Plants growth

Plants growth parameters are reported in Table 5, for both the experimental and the control pots.

Biomass development is a good indicator of the plant health which reflects a balanced availability of nutrients and the absence of inhibitory effects by toxic substances. Roots development, either in terms of mass or length, allows the evaluation of the soil and of the phytotreatment performance capacity of each individual plant.

A significantly reduced roots development was observed in rapeseed compared to the corresponding control plants, both in terms of mass and length. Total biomass was approximately 21% of the biomass developed by the control plants,

Table 4 – Value range of the concentrations (expressed as mg/L) of the main analytical parameters describing the quality of water feedings during the different phases of the research.

Phases	COD	TKN	$N\text{-}NO_3$	Ptot	Cl^-	SO_4^{2-}	TS	VS	MBAS
Acclimatization	5 ± 1.2	0.3 ± 0.1	41.0 ± 8.8	3.1 ± 0.2	3.7 ± 0.2	48.0 ± 14	440 ± 11	150 ± 2	–
	23 ± 9	0.9 ± 0.2	0.07 ± 0.03	3.3 ± 0.1	13.4 ± 2.4	12.0 ± 1.6	278 ± 18	147 ± 3	0.13 ± 0.02
Phase I	48 ± 17	1.5 ± 0.4	0.01 ± 0.10	3.1 ± 0.1	27.6 ± 1.1	26.0 ± 3.3	401 ± 10	133 ± 6	0.30 ± 0.02
Phase II	528 ± 49	4.5 ± 1.6	0.60 ± 0.10	4.5 ± 0.7	29.8 ± 8.1	19.5 ± 3.7	703 ± 38	499 ± 69	57.59 ± 25.98
Phase III	530 ± 48	7.8 ± 2.3	0.60 ± 0.10	4.9 ± 0,8	31.4 ± 8.2	19.6 ± 3.7	711 ± 40	502 ± 70	57.53 ± 25.95
Phase IV	540 ± 47	17.7 ± 4.5	0.60 ± 0.10	5.9 ± 1.9	36.1 ± 8.3	20.3 ± 3.8	737 ± 41	512 ± 73	57.36 ± 26.00

COD: chemical oxygen demand; TKN: total Kjeldahl nitrogen; MBAS: Methylene Blue active substances; VS: volatile solids; TS: total solids.

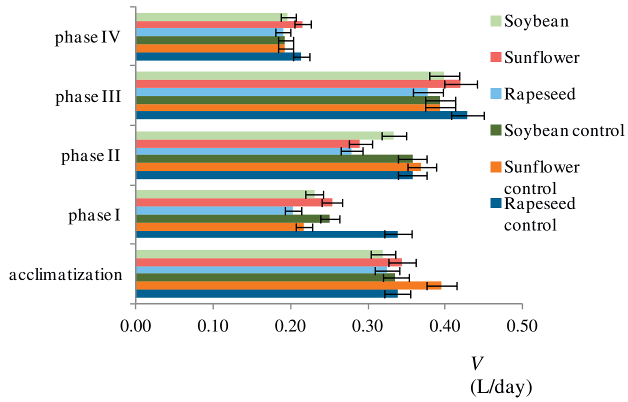

Fig. 3 – Influent volume of water provided daily in the four experimental phases for the three different tested plant species.

while the root biomass was even lower (13%). This is a clear indication that rapeseed is not a suitable species for the phytotreatment of the kinds of wastewater tested.

Sunflower produced less biomass than the control, while roots (in terms of length and weight) grew 10% more. Total biomass and the roots weight of soybean corresponded to approximately 60% that observed in control plants, indicating an equally distributed growth between root and shoot. However, root length was approximately 20% higher than in controls.

Generally, even when the total amount of nutrients added through wastewater irrigation was comparable to the amount recommended for optimal plant growth (Güsewell, 2004; Hoagland and Arnon, 1950), plants irrigated with wastewaters developed a lower biomass than the corresponding controls.

These results are partially in contrast with those obtained in similar experiments both for sunflowers (Khan et al., 2009) and other plant species (Gandini, 2004), where vegetative growth was enhanced by irrigation with grey, yellow and kitchen waters. These divergent results could be linked to the low content of nitrogen in the greywaters, as highlighted earlier. This fact might have resulted in a shortage of nutrients at the beginning of our experiment (phase I), when only greywaters were fed, which inhibited plants growth, as observed in previous studies (Güsewell, 2004; Jones et al., 2011). This early impairment in plant growth was not recovered in the following phases, despite an increase in nutrient loading with addition of yellow and kitchen waters.

2.3. Removal efficiency

Fig. 4 describes graphically the variation in time of the loading of nutrients and COD in the feeding, concentrations of the same parameters in the outflow streams and removal yields, as observed throughout the different phases. Loadings are expressed in terms of surface load (mg/m^2/day) in order to allow comparison with literature data. Removal yields (η, %) were obtained by computing the input and output loads:

$$\eta = (V_{in} \cdot C_{in} - V_{out} \cdot C_{out})/(V_{in} \cdot C_{in}) \cdot 100\% \quad (1)$$

where: V_{in} (L/week) = influent volume; V_{out} (L/week) = effluent volume; C_{in} (mg/L) = influent concentration; C_{out} (mg/L) = effluent concentration.

High nitrogen removal efficiency (>80%) was observed throughout the experimental period. In phase I nitrogen load was found to be quite low, (3–20 mg N/m^2/day). While this produced a negative effect, as observed earlier, on the development of plant biomass, high removal efficiencies were achieved. In phases II, III and IV the N load was gradually increased from 20 to 198 mg N/m^2/day for sunflower and soybean, and from 10 to 140 mg N/m^2/day for rapeseed, due to the contribution of yellow water (Table 4).

The different values of the nitrogen load observed for the three species were the result of the different water demand of the individual plants (Fig. 3).

Throughout the last three phases, with the exception of a slight drop at the end of phase II, removal efficiencies remained stable, higher than 90%, indicating a positive and rapid response of the system to the increase of nitrogen loading. Nitrogen concentration in the outflow was constantly below 10 mg/L.

Throughout the entire experiment, mean phosphorous load values ranged between 30 mg P/m^2/day (sunflower) and

Table 5 – Total biomass, biomass and length of roots measured in the individual plants at the end of the research period, and variation η (%) between treated plants and respective controls.

Plant species		Rapeseed	Sunflower	Soybean
Total biomass	Test (g/pot)	7.56 ± 0.86	4.41 ± 0.78	7.79 ± 0.23
	Control (g/pot)	36.10 ± 2.69	6.51 ± 1.47	11.99 ± 2.02
	Test/Control (%)	21	68	65
	η (%)	−79	−32	−35
Root mass	Test (g/pot)	3.13 ± 0.23	0.49 ± 0.08	0.99 ± 0.03
	Control (g/pot)	24.40 ± 1.55	0.45 ± 0.12	1.71 ± 0.17
	Test/Control (%)	13	110	58
	η (%)	−87	10	−42
Main root length	Test (g/pot)	7.24 ± 0.88	7.74 ± 1.25	8.38 ± 1.55
	Control (g/pot)	11.20 ± 1.20	7.00 ± 0.93	6.75 ± 1.13
	Test/Control (%)	65	111	124
	η (%)	−35	11	24

Fig. 4 – Variation in time of the feeding load, the outflow concentrations and of the removal yields of total nitrogen (N_{tot}) (a), total phosphorous (P_{tot}) (b) and COD (c) along the experiment for the three plant species.

35 mg P/m^2/day (rapeseed and soybean) being within the phosphorous plant demand (Holmes, 1980). Removal efficiencies were very high for all plant species, with a concentration in the outflow below 1 mg P/L. This clearly indicates that this nutrient was almost completely removed by the system.

During phase I, due to the low inflow COD concentrations (Table 4), the removal efficiencies were very high, with a COD output constantly below 10 mg/L. From phase II the COD concentration was increased by the addition of yellow and kitchen wastewaters. In phases III and IV COD load in the inflow fluctuated as a result of the high variability of the quality of kitchen waters. Nevertheless, COD in the outflow remained below 100 mg/L.

The COD load in the different phases ranged between 200 mg O_2/m^2/day (Acclimatization and Phase I) and 2000–4000 mg O_2/m^2/day (Phases II, III and IV).

The good COD removal efficiency is related to the synergic effects of the chemical, physical and biological processes occurring in the plant-substrate system (sedimentation, filtration, adsorption in the substrate, biodegradation of the organic matter and uptake by plant roots), as reported by Duggan (2005).

Generally, COD, N and P removal rates are higher than those reported in previous studies (Keffala and Ghrabi, 2005; Khan et al., 2009). Input and output concentrations and related removal efficiencies with regard to parameters other than nutrients and COD are reported in Table 6.

The efficiency of MBAS removal was very high (more than 95%) for all plant species, even in the presence of high input concentrations (up to 111 mg/L in phase II) (Table 6). Similar findings were recently reported by Ramprasad and Philip (2016).

Copper and iron are reported as they are deemed of interest due to the detection of concentrations present in input wastewaters (see Table 1). For both heavy metals, which were detected in significant concentrations, removal rates were very high particularly for sunflower. Outflow concentrations lower than 0.01 mg/L for Cu and 0.5 mg/L for Fe were always achieved.

Although the concentrations of chloride and sulphate increased in the feeding due to the increasing percentage of yellow water during the different study phases (Tables 1 and 4), no effects on plant growth were detected. As expected (Ouyang, 2013), removal rate of chloride and sulphate was limited; in particular, soybean plants were found to be the less efficient to increasing chloride load but displayed the best performance in sulphate removal. Removal efficiencies for

Table 6 – Input and output concentrations of several parameters and removal efficiency rates observed for the different plant species.

Parameter	Species	IN		OUT		η (%)
		Min–Max	Average	Min–Max	Average	
Cl^- (mg/L)	Rapeseed	27.6–44.4	30.8	23.6–28.5	24.4	21
	Sunflower			22.9–27.7	23.6	23
	Soybean			26.1–31.5	26.9	13
SO_4^{2-} (mg/L)	Rapeseed	19.5–29.3	21.8	15.1–16.9	15.8	23
	Sunflower			13.7–15.4	14.4	30
	Soybean			13.2–14.8	13.9	32
TS (mg/L)	Rapeseed	401–778	732	384–542	485	61
	Sunflower			330–440	399	79
	Soybean			520–684	587	68
VS (mg/L)	Rapeseed	133–585	505	118–280	223	77
	Sunflower			98–160	124	90
	Soybean			136–402	281	79
Cu (μg/L)	Rapeseed	27.0–82.9	112.0	10.0–12.0	11.0	90
	Sunflower			<10.0	10.0	93
	Soybean			<10.0	10.0	93
Fe (μg/L)	Rapeseed	31.0–1150.0	647.0	10.0–404.0	160.0	86
	Sunflower			10.0–510.0	25.0	100
	Soybean			10.0–488.0	170.0	99
MBAS (mg/L)	Rapeseed	0.30–83.57	43.5	0.1–1.3	0.8	98
	Sunflower			0.1–1.5	0.9	98
	Soybean			0.1–1.3	0.8	98

IN: input concentration; OUT: output concentration; η: removal efficiency; MBAS: Methylene Blue Active Substances.

TS and VS throughout the different research phases exceeded 60%–70%.

2.4. Nitrogen balance

At the end of the entire experiment a total nitrogen balance for each pot was calculated on the basis of the following equation:

$$N_{in} = N_{out} + Np + Ns + Nb \quad (2)$$

where, N_{in} (mg) = total mass of nitrogen entering the pot plant–soil system, nitrogen input as sum of TKN and $N\text{-}NO_3$ loads provided throughout the entire experiment ($N\text{-}NO_2$ was negligible); N_{out} (mg) = total mass of nitrogen in the outflow; Np (mg) = amount of nitrogen accumulated in the plant tissue; Ns (mg) = nitrogen accumulated in the substrate; Nb (mg) = balancing term for closing the equation. This term takes into account the nitrogen gaseous loss.

The mean values (averages of four replicates) of the nitrogen balance terms are reported in Table 7.

The input values indicate that the total amount supplied by wastewater irrigation is close to the common nitrogen demand of each plant species (Holmes, 1980) being slightly higher (rapeseed and soybean) or lower (sunflower) with respect to the amount supplied to the corresponding controls. Despite this evidence, N plant uptake for each individual plant was lower than in the controls. This is particularly evident for rapeseed as a consequence of the larger biomass development observed in the control (Table 5).

A graphical description of the relevance of the different whereabouts of the nitrogen mass provided with the inflow is given in Fig. 5. It clearly shows how the soil plays the most important role in phytotreatment removal, as observed in several other studies, connected in particular to the bacterial metabolism around the root zone (Griffiths and Robinson, 1992).

Table 7 – Mean values of the nitrogen balance terms measured at the end of the entire experiment for each individual plant–soil system (pot). Data are the averages of four replicates and are expressed both as mg/pot and percentage of Total N input. Surface pot is 0.045 m^2.

Species	N_{in}	N_{out}		Np		Ns		Nb	
	mg	mg	%	mg	%	mg	%	mg	%
Rapeseed	860 ± 54	1.5 ± 1.1	0.2	80 ± 14	9	780 ± 88	91	−1.5 ± 0.3	–.2
Sunflower	630 ± 38	0.7 ± 1.2	0.1	60 ± 21	10	570 ± 75	91	−0.7 ± 0.9	−0.1
Soybean	850 ± 77	1.3 ± 0.6	0.2	170 ± 36	20	670 ± 53	79	8.7 ± 1.8	1.0
Rapeseed control	780 ± 10	1.0 ± 0.4	0.1	140 ± 23	18	680 ± 43	87	−41.0 ± 7.9	−5.3
Sunflower control	730 ± 12	2.1 ± 1.0	0.3	70 ± 31	10	590 ± 48	81	67.9 ± 13.7	9.3
Soybean control	710 ± 18	1.1 ± 0.3	0.2	260 ± 45	37	460 ± 37	65	−11.1 ± 2.3	−1.6

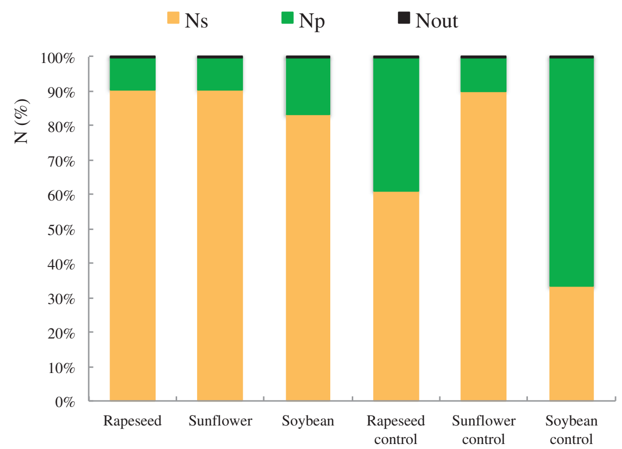

Fig. 5 – Balance of whereabouts of the total nitrogen mass (N_{in}) entering the soil–plant system. Ns = nitrogen in soil, Np = nitrogen uptake by plants, N_{out} = residual nitrogen in the outflow. All terms are expressed as % of N_{in}.

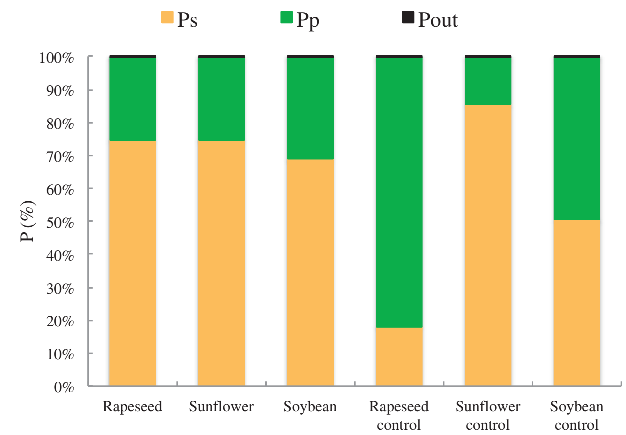

Fig. 6 – Balance of whereabouts of the total phosphorous mass (P_{in}) entering the soil–plant system. Ps = phosphorous in soil, Pp = phosphorous uptake by plants, P_{out} = residual phosphorous in the outflow. All terms are expressed as % of P_{in}.

Similar to nitrogen balance, a mass balance for phosphorous has been drawn and mean values are reported in Table 8:

$$P_{in} = P_{out} + Pp + Ps + Pb \quad (3)$$

where, P_{in} (mg) = total mass of phosphorous entering the pot plant–soil system; P_{out} (mg) = total mass of phosphorous in the outflow; Pp (mg) = phosphorous plant uptake measured as total mass of phosphorous accumulated in the plant tissue; Ps (mg) = phosphorous accumulated in the substrate; Pb (mg) = balancing term for closing the equation.

Contrary to nitrogen, the total phosphorous amount supplied by wastewater irrigation is slightly lower than amount provided to the corresponding control pots. Treated plants accumulated less P than their respective controls (Table 8). The graphical representation of the whereabouts of phosphorous in the inflow once again highlights the fundamental role of the soil, which appears however less important than for nitrogen (Fig. 6).

3. Conclusions

The basis underlying the investigation was a pot trial comprising three species of oleaginous plants (rapeseed, soybean and sunflower), aimed at assessing their ability to treat grey, yellow and kitchen wastewaters and at calculating, with respect to the plant–soil system, a balance of the whereabouts of the nutrients (N and P) loads supplied with the wastewaters. The investigation was divided into four distinct phases using different mixtures of the three wastewaters with the aim of progressively increasing nutrients and organic content in the irrigation water.

The following conclusions could be drawn: (1) Rapeseed, soybean and sunflower plants treated with wastewaters presented a biomass development lower than the controls. The reduced vegetative growth was mainly due to a general scarcity of available nutrients for plants at the beginning of the growth stage (phase I of the study), when plants were fed with grey waters only. (2) The addition of yellow waters increased the nitrogen concentration in feed, determining a positive response of plants both in terms of growth and removal efficiency. (3) The removal efficiencies for N, P and COD remained higher than 80% for all plant species throughout the period. Sunflower plants induced the highest removal rates, whilst rapeseed plants featured the lowest removal rates and the highest biomass reduction. (4) The most crucial finding is related to the identification of an optimal combination of source-separated wastewaters and nutrient-loaded waters (kitchen water and yellow water), with the aim of achieving a satisfactory degree of plant growth and phytotreatment performance. (5) The removal mechanisms

Table 8 – Mean values of the phosphorous balance terms measured at the end of the entire experimental period for each individual plant–soil system (pot). Data are the averages of four replicates and are expressed both as mg/pot and percentage of Total P input.

Species	P_{in}	P_{out}		Pp		Ps		Pb	
	mg	mg	%	mg	%	mg	%	mg	%
Rapeseed	69 ± 15	0.1 ± 0.1	0.1	12 ± 3	17	59 ± 8	86	−2.0 ± 0.6	−3.0
Sunflower	53 ± 18	0.1 ± 0.1	0.2	10 ± 3	19	40 ± 3	76	2.9 ± 2.1	5.5
Soybean	71 ± 14	0.1 ± 0.3	0.1	18 ± 2	25	50 ± 7	70	2.9 ± 0.8	4.1
Rapeseed control	82 ± 3	0.1 ± 0.2	0.1	31 ± 4	38	54 ± 4	66	−3.1 ± 1.1	−3.8
Sunflower control	78 ± 3	0.2 ± 0.1	0.3	18 ± 2	23	58 ± 5	74	1.8 ± 0.9	2.3
Soybean control	76 ± 2	0.1 ± 0.2	0.1	39 ± 4	51	40 ± 9	53	−3.1 ± 1.7	−4.1

involve complex interactions between chemical, physical and biological processes.

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