



PHYTOREMEDIATION OF ARSENIC BY *Phragmites australis*, *Colocasia esculenta* AND *Canna indica* USING A NOVEL SEMI-HYDROPONICS SYSTEM

Mohd Kaifiyan*, Fareed A. Khan and Mohd Irfan Naikoo*

Plant Ecology and Environment Laboratory, Department of Botany, Aligarh Muslim University,
Aligarh – 202 002, Uttar Pradesh (India)

*e-mail: mohdkaifiyan94@gmail.com, mdirfanmsbo@gmail.com

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ABSTRACT

Hydroponics system is a potential and cost-effective technique for wastewater treatment. Aquatic macrophytes are more efficacious material for phytoremediation due to their physiological and morphological properties. We designed a set of three hydroponics (PVC pipes) interconnected in series so as the inlet of first hydroponic was connected from tank containing arsenic (As) contaminated wastewater and its outlet with the second as its inlet and outlet of second with the third one. In each hydroponic, 6 pots (filled with soil and compost and referred as semi-hydroponics systems viz., SHS-1, SHS-2 and SHS-3) with pre-established single individual of *Phragmites australis*, *Colocasia esculenta* and *Canna indica* were placed in SHS-1, SHS-2 and SHS-3, respectively. Wastewater was retained in each SHS for 10 days (hydraulic retention time); and passed from 1st to 2nd and then to 3rd one in series at every 10 days intervals. The growth performances of selected macrophytes were compared with and control plants maintained in freshwater hydroponics. Wastewater significantly enhanced shoot length, proline content, chlorophyll content and sugar content of leaf tissue of all the studied macrophytes. The plants and soil sediments absorbed As from the waste water efficiently and retained amount of As in phytoremediated spent wastewater from third semi-hydroponic system (SHS-3) was below limit of quantification of ICP-MS. The semi-hydroponic system in this experiment was found to be an efficient, cost-effective, easy to be installed and globally applicable phytoremediating technique.

Keywords: Arsenic, *Canna indica*, *Colocasia esculenta*, *Phragmites australis*, phytoremediation, semi-hydroponics, wastewater

INTRODUCTION

Arsenic (As) contamination has received worldwide attention due to its high toxicity to plants, animals including humans as inorganic arsenicals are proven carcinogens to human (Shams *et al.*, 2022). As in wastewater mainly comes from arsenic-solidified residues, fossil fuel combustion, ore mining, smelting of non-ferrous metals, preparation of As containing drugs, volcanic eruption and the use of pesticides (Lifa *et al.*, 2019). The wastewater from several sources (industrial effluent, factories waste and domestic sewage) possesses significant amounts of toxic heavy metals including As, which can be retained in soil (Ullah *et al.*, 2022), and percolate down to ground water (Bhardwaj *et al.*, 2020). As is readily absorbed by plant roots and reaches other plant parts through nutrient uptake; and are subsequently transferred to the food chain (Zhao *et al.*, 2010; Naikoo *et al.*, 2019; Bhardwaj *et al.*, 2020). As occurs in inorganic forms in the environment and accumulates in organic form in plant organs after their uptake (Abbas *et al.*, 2018; Khan *et al.*, 2021). Among the various chemical forms, arsenite (AsIII) and arsenate (AsV) are easily absorbed by the plant roots (Abbas *et al.*, 2018; Khan *et al.*, 2021). As accumulation in plants reduces several growth parameters including seed germination, root growth, biomass and chlorophyll content and induce swelling of root tips (Zemanová *et al.*, 2021; Zaid *et al.*, 2022).

The utility of hydroponically grown plants in toxicological studies is reliably established, in addition to its application in organic cultivation of native and exotic vegetables and ornamental plants (Naikoo *et al.*, 2020; Bhattacharya and Mandal, 2022). The hydroponic culture of ornamental plants has been suggested for the phytoremediation of heavy metals (Khan *et al.*, 2021). Hydroponic system can be defined as a technique of growing plants without the use of soil (Bello *et al.*, 2018; Mustafa *et al.*, 2021). But in present study, soil was used as a substrate medium for rooted-macrophytes and lateral roots were in direct contact with waste water in hydroponics pipes that facilitated the uptake of nutrients and heavy metal (As) from wastewater. In the context of wastewater treatment, plants are grown using nutrient-rich wastewater in hydroponic wastewater treatment systems (Bello *et al.*, 2018; Mustafa *et al.*, 2021). This procedure is considered an indispensable step in phytoremediation of wastewater, in which plant roots are responsible for degradation of excess nutrients and uptake of heavy metals.

Wastewater treatment through plants in hydroponic system is one of the promising and sustainable bioremediation methods for municipal-, agricultural- and industrial-wastewater. The small space requirements have triggered the applications of hydroponics for the cultivation of different species of plants to facilitate biological treatment of wastewater (Mustafa *et al.*, 2021). Most of the study on hydroponic include only single plant species which either of free floating or rooted (Baweic *et al.*, 2018; Gebeyehu *et al.*, 2018); but in the present study, three different species of rooted macrophytes were grown in hydroponics in series which phytoremediate wastewater in succession that has created uniqueness in this hydroponics system. The main objective of this study was to evaluate the semi-hydroponics as an alternative efficient technique to remediate As-laden wastewater; and to check the phytoremediation efficiency and tolerance capacity of the selected macrophytes. In present study, we applied a specifically designed simple and cost-effective semi-hydroponic technique for a very effective removal of As from wastewater by phytoremediation through *P. australis*, *C. esculenta* and *C. indica* growing in a series.

MATERIALS AND METHODS

Designing of semi-hydroponic systems and their interconnections

Customized semi-hydroponic systems, consisting of six PVC pipes (2 m long, 160 mm dia.) closed with end caps at both ends, were designed for the experiment. In each pipe, 6 holes of 15 cm dia. were drilled to insert PVC pots of 15 cm dia. In each end cap, one 25 mm bibcock-controlled inlet (on upper side of the end cap) and one outlet (on lower side of the end cap) were fixed. Two sets of hydroponic systems (one for phytoremediation of wastewater and other as freshwater reference/control set), each of three pipes were prepared and placed horizontally on the two slanting customized angle iron stands so as the bottom of the 1st pipe was above the level of upper surface of the next pipe in series and similar levels were maintained between 2nd and 3rd hydroponic pipes.

Experimental setup

Two weeks old saplings of *Phragmites australis*, *Colocasia esculenta* and *Canna indica* were collected along with roots and rhizome from local wetlands of Aligarh, Uttar Pradesh (India). Plants were washed carefully with tap water to remove soil sediments and one individual of each species was transferred to 15 cm PVC pots each filled with 1.5 kg soil and compost (3:1 ratio) and kept in 25 L fresh water in hydroponics pipes for 15 days to overcome transplant injuries and establish in pots. Fifteen days later, in phytoremediation series, fresh water of the 1st hydroponic pipe holding 6 pots of *P. australis* was replaced with pre-treated wastewater. The wastewater used in this experiment was collected from Sewage Treatment Plant, AMU, Aligarh. Ten days later, the fresh water of 2nd hydroponic (consisting of 6 pots of *C. esculenta*) was replaced with the partially spent wastewater of the 1st hydroponic (the 1st hydroponic pipe was re-loaded with pre-treated source wastewater). Again after 10 days, the fresh water of 3rd hydroponic (consisting of 6 pots of *C. indica*) was replaced with the 2nd stage spent wastewater of 2nd hydroponic. The 3rd stage spent wastewater of 3rd pipe was finally collected in the end tank. A similar series of hydroponics of all selected species (in the same order of plant species) was maintained with fresh water as reference/control series. The

process was continued for 150 days with regular exchanges of wastewater at 10 days intervals in series from source wastewater tank to first hydroponic, 1st to 2nd, 2nd to 3rd hydroponic and finally collected in the end tank of 3rd stage phytoremediated spent wastewater. At every 10 days interval, 100 mL water sample of each hydroponic was collected and stored in PVC vials. Non-destructive growth parameters of each species were recorded at 30 days intervals up to 150 days. Six replicates of each plant species were maintained in each semi-hydroponics system.

Plant growth parameters

Shoot length of each species was measured with a meter scale at 30 days interval. Each harvested plant was weighed separately using electronic balance (AH220A, AHN Germany) to record their fresh weight. For dry weight estimation, plant samples were placed in a drying oven at 70°C for 72 h before measuring their weights.

Physiological parameters

The total chlorophyll and sugar contents in leaves were estimated as per Arnon (1949) and DuBois *et al.* (1956), respectively; while proline (indicator of plant stress) contents in leaves was estimated by standard protocol given by Bates *et al.* (1973); and expressed in mg g⁻¹ fresh tissue.

Arsenic estimation

The plants of all the three selected macrophytes were harvested after 150 days exposure to wastewater for arsenic estimation. Dry powdered plant material (1 g), dried milled soil (1 g) and wastewater (100 mL) samples were pretreated with 60% nitric acid (HNO₃) for 24 h and then heated to 80°C for 2 h. The 70% perchloric acid (10 mL) was added to each sample, and the mixture heated to 200°C until the solution became clear. The samples were filtered through Whatman filter paper No. 44 and the element contents analyzed using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7000x series).

Data analysis

Data were statistically analyzed using SPSS (version 23) for mean, standard deviations and least significant difference among the variables. The regression analysis was done using Ms Excel.

RESULTS AND DISCUSSION

Plant growth

The shoot length was recorded at varying growth stages of all three selected plants grown in fresh water and wastewater hydroponics. The shoot growth of all the selected aquatic plants was relatively faster in wastewater hydroponics as compared to their reference plants (Fig. 1A-C). The linear regression line plotted between the shoot length and growth stages (days) of *P. australis* and *C. indica* grown in wastewater had relatively strong linear correlations (R^2) between shoot length and growth stage, followed by R^2 in freshwater hydroponics indicating that both these plants had greater adaptability in nutrient rich heavy metal-laden wastewater (Fig. 1A-C). The concave curvilinear relationship between the similar variables of *C. esculenta* had stronger correlation in wastewater hydroponics as compared to the control plants (Fig. 1 B). Alghobar and Suresha (2016) have reported that untreated wastewater irrigation enhanced plant height of rice crop as compared to ground water irrigation. Petousi *et al.* (2019) reported that wastewater irrigation enhanced the plant growth of grapevines.

Proline content

The proline contents in all the three aquatic macrophytes grown in wastewater hydroponics were higher than their respective reference plants grown in freshwater hydroponics (Fig. 1 D-F). The regression lines plotted between proline content and growth stages of *P. australis*, *C. esculenta* and *C. indica* grown in fresh and wastewater were linear (Fig. 1 D-F) with strong and squared positive correlation coefficients (R^2). High proline content as found in the present experiment may be an outcome of plants defensive strategies against stresses of heavy metal (Antolín *et al.*, 2010). In present study, the excessive proline accumulation indicating strong resistance to arsenic and evolved

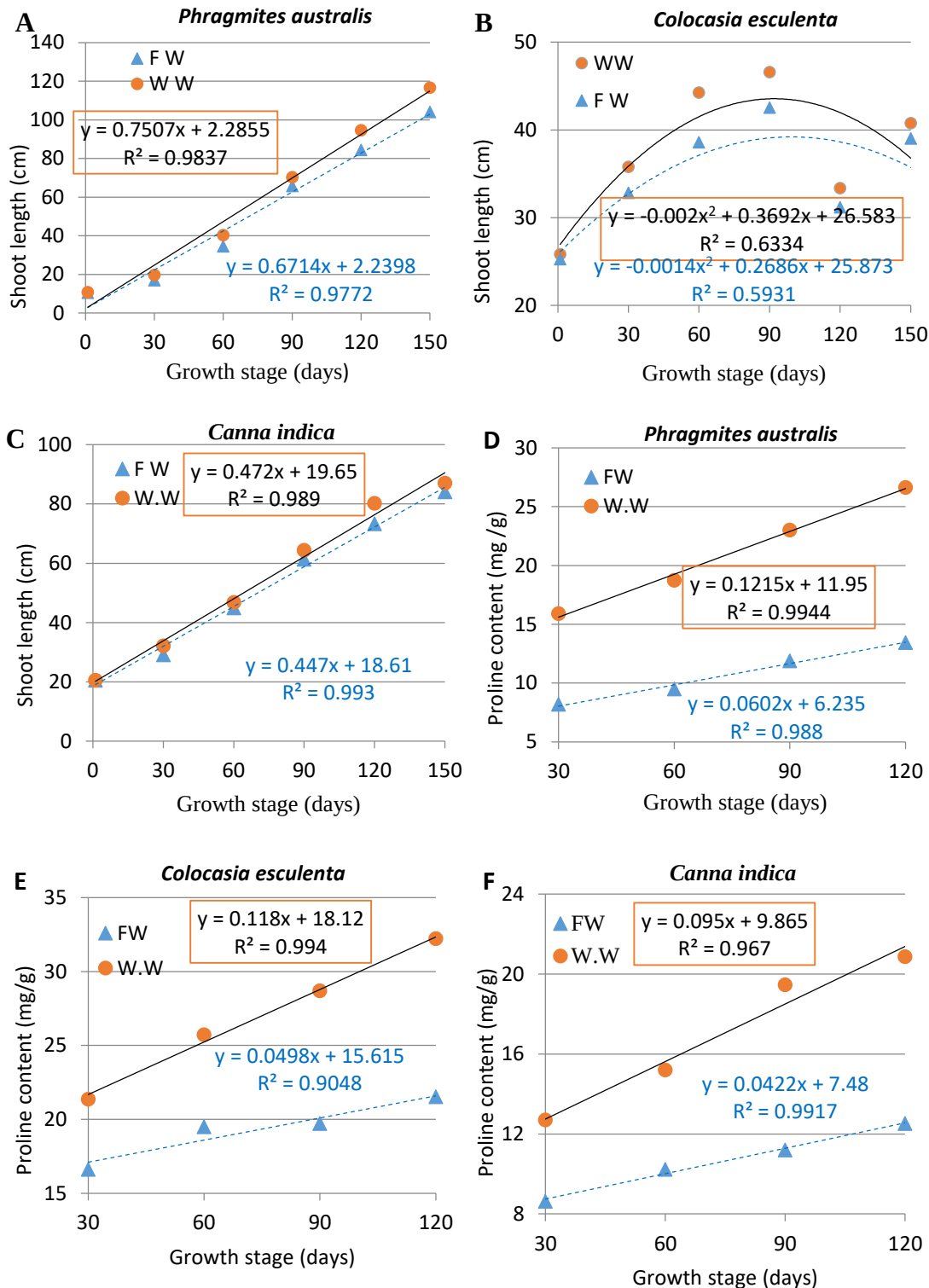


Fig. 1: Linear and curvilinear regression lines, equations and R^2 (squared correlation coefficients) between shoot length (cm), proline content (mg g^{-1} fresh leaf tissue) and growth stages of *Phragmites australis* (A, D), *Colocasia esculenta* (B, E) and *Canna indica* (C, F); grown in fresh water (FW) and wastewater (W.W) hydroponics

abilities of driving benefits from excessive nutrients to increase its tissue systems in the form of excessive growth to sequester arsenic in its cell apoplast.

Plant biomass (fresh and dry weight)

Total fresh and dry weights of *P. australis*, *C. esculenta* and *C. indica* grown in fresh water and wastewater did not show any significantly difference ($p > 0.05$) [Table 1]. Plant biomass was found

Table 1: Fresh and dry weight (g) of *Phragmites australis*, *Colocasia esculenta* and *Canna indica* at 150 days growth in fresh water and wastewater hydroponic system

Hydroponic type	Fresh and dry weight (g) at 150 days					
	<i>Phragmites australis</i>		<i>Colocasia esculenta</i>		<i>Canna indica</i>	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
Freshwater	122.2 ± 21.0	12.2 ± 2.6	110.5 ± 30.9	14.9 ± 3.6	108.9 ± 6.9	15.7 ± 2.9
Wastewater	126.8 ± 19.8	12.9 ± 2.2	114.6 ± 18.3	16.1 ± 3.9	112.6 ± 6.2	16.6 ± 2.5
t-test	0.399, NS	0.473, NS	0.278, NS	0.530, NS	0.966, NS	0.526, NS

NS = not significant ($p > 0.05$)

an important measure to assess various environmental stresses and overall health in the presence of heavy metals (Naikoo *et al.*, 2019, 2020; Nicole *et al.*, 2021). Despite no significant effect of arsenic on plant fresh and dry weight, the present study agrees with those of Lucia *et al.* (2011) who recorded significant effects of heavy metals on plant growth, tissue concentration and their uptake by sunflower in soil. Vithanage *et al.* (2012) and Shyni *et al.* (2014) have reported that lower concentrations of As insignificantly affected the fresh and dry weight of the treated plant, due to tolerance behavior of the plant which is in line with our study. This justifies that As uptake have no significant effect on plant biomass. The selected macrophytes showed high tolerance to As and, therefore, are used in phytoremediation studies.

Chlorophyll content

Chlorophyll contents (mg g^{-1} fresh leaf) in all three selected phytoremediating plants grown in wastewater hydroponics were relatively higher than in freshwater hydroponics at all the growth stages (Fig. 2 A-C). The squared correlation coefficients between the amounts of chlorophylls and age of all three selected macrophytes both in wastewater and freshwater were positive and strong (> 0.9). The patterns of regression lines between these variables were also linear (Fig. 2 A-C) in both wastewater and freshwater treated macrophytes indicating the increase in chlorophyll contents in all the three macrophytes (*P. australis*, *C. esculenta* and *C. indica*) were not adversely affected by heavy metals present in wastewater instead, the nutrients in wastewater consistently increased total chlorophyll contents with plant age (Fig. 2 A-C). The macronutrients in wastewater provide cofactors to the enzymes necessary for the synthesis of amino acids, proteins and carbohydrates and so resulted in the increase in total proline, carbohydrates and chlorophyll contents in *Lens culinaris* and *Cicer arietinum* leaves (Rija *et al.*, 2005). Wastewater contains significant amounts of organic and inorganic nutrients (Wang *et al.*, 2012). In contrast, the toxic levels of mineral elements in the wastewater resulted in a significant decline in chlorophyll content in leaf tissues of *Triticum aestivum* (Hajihashemi *et al.*, 2020).

Sugar contents

Total sugar contents (mg g^{-1} fresh leaf tissue) of all three plants significantly ($p < 0.05$) increased with growth stage both in freshwater and wastewater hydroponics (Fig. 2 D-F). Squared correlation coefficients (R^2) of *P. australis*, *C. esculenta* and *C. indica* between total sugar contents and growth stages were stronger and positive in wastewater than in freshwater hydroponics (Fig. 2 D-F) indicating direct dependence of this growth factor on nutrients in wastewater than any adverse effect of heavy metals. The regression lines and equations best fitting to the variables (sugar content in leaves vs growth stages) were also linear (Fig. 2 D-F) in all the three selected species grown in freshwater and wastewater hydroponics. Application of treated wastewater enhanced sugar contents in the leaves of *Brassica nigra*, *Colocasia esculenta* and *Raphanus sativus* (Gupta *et al.*, 2010). The increase of sugar content in leaves on sewage water application may be a direct outcome of either faster rate of photosynthesis that phloem unloading or higher both together loading to higher plant biomass as noted in the present experiment (Zeid and Ghate, 2007).

Arsenic (As) accumulation

The As concentration in source wastewater was 1.57 mg L^{-1} and in garden soil with compost filled in each pot was initially below ICP-MS limit of quantification (BLQ). The freshwater was used as control in separate set of hydroponics free from any contamination of As and nutrients. As concentrations in phytoremediated spent wastewater (composite samples of 150 days) of outlets of 1st

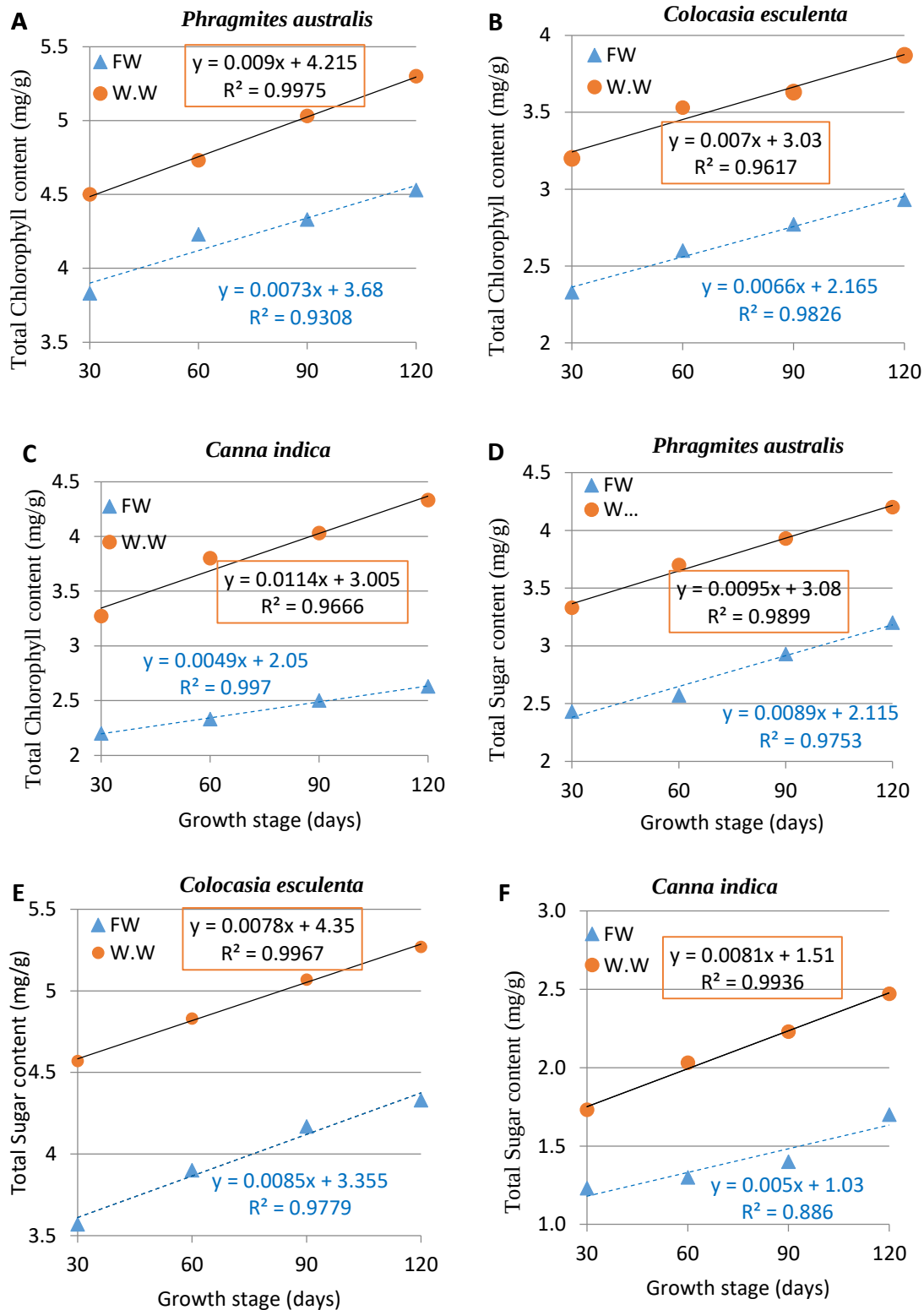


Fig. 2: Linear regression lines, equations and R^2 (squared correlation coefficients) between total chlorophyll content (mg g^{-1} fresh leaf tissue), Total sugar content (mg g^{-1} fresh leaf tissue) and growth stages of *Phragmites australis* (A, D), *Colocasia esculenta* (B, E) and *Canna indica* (C, F), grown in freshwater and wastewater hydroponics

semi-hydroponic system (SHS-1) were 0.15 mg L^{-1} and BLQ in outlets of SHS-2 and SHS-3. As concentrations in soil of pots of S.H.S-1, S.H.S-2 and S.H.S-3 were 0.03 mg kg^{-1} , 0.02 mg kg^{-1} and BLQ, respectively (Table 2). As accumulations in whole dry plants of *P. australis*, *C. esculenta* and

C. indica of SHS-1, SHS-2 and SHS-3 were 1.15 mg kg⁻¹, 0.37 mg kg⁻¹ and BLQ, respectively (Table 1). Similarly, in varying experimental approaches, all the three macrophytes had effective phytoremediating abilities of heavy metals from contaminated water and soil (Bello *et al.*, 2018; Thathong *et al.*, 2019). Among these three species, *P. australis* was specifically well adapted to a wide range of toxicities caused by several heavy metals and metalloids including As (Bello *et al.*, 2018; Nicole *et al.*, 2021). In different organs of *P. australis*, translocation of heavy metals with highest element concentrations was in the order: roots > rhizomes > leaves > stems (Zhang *et al.*, 2021). *C. indica* is a good accumulator of Cd, Pb, Ni (Subhashini *et al.*, 2014)

Table 2: ICPMS determined concentration of arsenic (As) in wastewater (mg L⁻¹, at source, outlets of respective hydroponics), soil sediment (mg kg⁻¹ dry soil) and 3 selected macrophytes (mg kg⁻¹ dry plant) grown in 3 semi-hydroponic systems (SHS 1-3) each containing 25 L of wastewater with 10 days hydraulic retention time in respective hydroponics run for a total of 150 days.

Heavy metals	Source		SHS-1	SHS-2	SHS-3
	(Inlet-1)		<i>Phragmites australis</i>	<i>Colocasia esculenta</i>	<i>Canna indica</i>
Arsenic (As)	WW	1.57	0.15	BLQ	BLQ
	Soil	BLQ	0.03	0.02	BLQ
	Plant	-	1.15	0.37	BLQ

WW = wastewater, SHS = semi hydroponic system, BLQ = below limit of quantification by ICP-MS

and later it was reported as efficient phytoremediator of As (Khan and Bano, 2016) and removed 9.9 to 40% As from soil. Similarly, Thathong *et al.* (2019) reported the role of *C. esculenta* in As removal in a pilot-scale constructed wetland water containing As concentration of 0.50 mg L⁻¹ and influent flow at 1.5 m³ day⁻¹. The As in water decreased from 0.485 to 0.054 mg L⁻¹ and *C. esculenta* was found responsible for As accumulation which support present study. *C. esculenta* take up a large portion of As from waste water and retained BLQ As in outlet water of SHS-2. High heavy metals uptake abilities of *P. australis* without adverse effects on its growth is attributed to the sequestration of trace metals in lignin and cellulose components of the expanded tissue systems as appears to be a species-specific evolved defense mechanism (Nicole *et al.*, 2021). The aerenchyma in the roots and rhizomes of wetland plants improve anaerobic conditions and remove hypoxic stress of the waterlogged plants besides other associated biogeochemical processes in sediments (Nicole *et al.*, 2021).

Conclusions: All three selected aquatic macrophytes studied in the present experiment had evolved traits to derive benefit from excessively nutrient rich sewage water to increase the tissue biomass for apoplastic uptake and accumulation of arsenic in these plants. *Phragmites australis* and *Colocasia esculenta* grown in two separate hydroponic 1 and 2, respectively, removed arsenic to the extent below the detectable limits of ICP-MS. Treatment of all three species in separate hydroponics spared the macrophytes from any inter-species competition through root exudates, if any. The wastewater used in the experiment was untreated and had adequate nutrients and thus, there was no nutrient deficiency in the medium of all three macrophytes.

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Conflict of interest: Authors declare that they have no conflict of interest regarding publication of this manuscript.

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