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RESEARCH ARTICLE

VEGETATION EFFECT UPON MACROINVERTEBRATE COMMUNITIES IN A VERTICAL-FLOW CONSTRUCTED WETLAND TREATING DOMESTIC WASTEWATER.

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Abstract

Constructed wetlands play an important role in wastewater pollutants removal. Thus, several investigations have been carried out about the different components of the system in order to acquire scientific knowledge on the pollutants removal processes that take place there. However, there is little knowledge about the biodiversity that can be found in these artificial environments, especially concerning the invertebrate community level. Our study investigate the effect of different plants species (*i.e. Andropogon gayanus, Chrysopogon zizanioides, Echinochloa pyramidalis, Pennisetum purpureum, and Tripsacum laxum*) upon macroinvertebrates communities and their abundance in the sediments of a vertical flow constructed wetland treating synthetic domestic wastewater. The experiment was performed in a pilot-scale composed of six beds constructed with bricks. Each bed was filled from the bottom to the top with 0.1 m of gravel covered with cloth and 0.6 m of white lagoon sand. Five beds were transplanted while one unplanted was used as control. 80 L of domestic synthetic wastewater was then applied on the beds intermittently during six months. All the five plant species have well developed, improving the removal of total nitrogen (TN), total phosphorus (TP) and total suspended solid (TSS) from domestic wastewater in the constructed wetlands. However, *P. purpureum* provided highest above ground biomass and favored the best TSS, COD, TN and TP removal efficiencies. As for macroinvertebrates, 12 taxa belonging to 12 families, 6 classes and 6 orders were recorded. The community structure was influenced by plant species. Macroinvertebrates were significantly more diversified in the planted beds than in the control. Insects dominated all the planted beds, while Oligochaeta were the most abundant in unplanted bed. The bed planted with *P. purpureum* housed the largest number of organisms and was follow by those planted with *T. laxum, E. pyramidalis, A. gayanus* and *C. zizanioides*, respectively. Following the vertical profile, macroinvertebrates abundance decreased from the upper layer to the bottom layers and only Oligochaeta (Lumbricidae) were recorded in all beds sediments layers of the CW.

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Introduction:-

Natural wetlands have historically been used as receiving bodies for effluent discharges. However, their use was more like a practical spill inducing the degradation of wetlands than an optimized treatment system. Constructed wetlands (CWs) for wastewater treatment were born from the improved knowledge of the functioning of natural wetlands, and Dr. Käthe Seidel was the first to experiment with this type of field in 1960, in Germany (Molle, 2012). This process, inexpensive, efficient and easy to perform compared to conventional sewage treatments systems (*e. g.*, activated-sludge systems), has been proven itself in various parts of the world in the protection of environment and populations health (Molle *et al.*, 2004, Lachat *et al.*, 2005, Vymazal *et al.*, 2006).

Depending on the type of design of the CWs, the applied wastewater flows essentially, vertically or horizontally into the beds during the treatment process. However, in conventional horizontal flow wetlands, the oxygen concentrations are limited (Nivala *et al.*, 2013) while unsaturated vertical-flow wetlands operate under a regime mainly aerobic. This condition should favor the oxygen transference rates (Schwager & Boller, 1997; Nivala *et al.*, 2013) and then can enhance the nitrification rates as well as the oxidation capacity in order to remove organic matter (Cooper, 1999; Zhang *et al.*, 2015). In addition, the use of vertical-flow CWs reduces environmental risks such as the release of odors and methane and the proliferation of mosquitoes, which are attributed to horizontal-flow CWs (Mander *et al.*, 2005).

Unlike most treatment processes that focus on a single treatment mechanism or a type of pollutant, wetlands use numerous interdependent symbiotic processes for concurrent removal of several different types of pollutants. Indeed, these process are summarized in a set of physical, chemical and biological mechanisms, more or less intertwined, via the main components of the system namely, plants, sediments and fauna (micro and macro fauna) living in the sediment (Poulet *et al.*, 2004). However, if sediment and plant can be manipulated during the construction of the wetland, the fauna develops itself naturally in the sediment of the beds (Stottmeister *et al.*, 2003, Vymazal, 2008).

Thus, in the quest for scientific knowledge on the pollutants (*i. e.*, organic matter, nitrogen and phosphorus, pathogenic germs, heavy metals, etc.) removal processes in CWs, several investigations have been carried out about the different components of the system (Wu *et al.*, 2015). However, if more attention has been paid to plants (Brix, 1994, 1997, Stottmeister *et al.*, 2003, Gagnon *et al.*, 2007, Coulibaly *et al.*, 2008a, 2008b, Vymazal, 2011, Wu *et al.*, 2011, 2013a, 2013b, Shelef *et al.*, 2013), to sediments (Prochaska & Zouboulis, 2006, Xu *et al.*, 2006, Calheiros *et al.*, 2008, Bruch *et al.*, 2011; Saeed & Sun, 2013), and microorganisms (Münch *et al.*, 2005, Tao *et al.*, 2006, Gagnon *et al.*, 2007), it has not been the same for macroinvertebrates. However, symbiotic relationships have been established between macroinvertebrates and microorganisms in the transformation of organic matter and nutrients in soils (Lavelle & Gilot 1994, Lavelle 1997).

Organic pollutants of wastewater and plant residues in the CWs bed sediments are composed mainly of biopolymers that assimilation by microorganisms proves difficult (Park *et al.*, 2009). Therefore, these are first ingested and decomposed by macroinvertebrates into simple elements, making them easily assimilated by microorganisms. In addition, macroinvertebrates can create tunnels in the beds substrate that could increase its aeration and the water infiltration rate (Ouattara *et al.*, 2009; 2011). In short, macroinvertebrates could create specific micro-habitats in the beds of constructed wetlands and favored the proliferation of the microorganisms, which are mainly involved in the degradation of pollutants in these systems (Lavelle, 1997; Deprince, 2003; Stottmeister *et al.*, 2003). Therefore, knowledge of the ecology of this fauna could help to evaluate the wetlands performance and better understand the purification mechanisms of the pollutants that take place there. However, studies in this area, such as those of Ouattara *et al.* (2009 ; 2011) are limited to the assessment of the effect of hydraulic load and that of a single plant species (*i.e.*, *Panicum maximum*), on the structure of the macrofauna in the constructed wetlands.

Objectives of this study were to (i) determine the performance of the vertical flow CWs using different plants species (*i.e.* *Andropogon gayanus*, *Chrysopogon zizanioides*, *Echinochloa pyramidalis*, *Pennisetum purpureum*, and *Tripsacum laxum*), to (ii) evaluate macroinvertebrates biodiversity, and to (iii) investigate the effect of the different plants species upon macroinvertebrates communities and their vertical distribution in the sediments of CWs.

Materials and methods:-

Experimental design:-

The experiment was conducted on a pilot-scale located at Nangui Abrogoua University (Abidjan, Côte d'Ivoire) characterized by a humid tropical climate with an average temperature varying between 25 and 33°C. The experimental setup consisted of six rectangular beds (length = 1.45 m, wide = 1.00 m, depth = 0.80 m and area of 1.45 m²) built cement according to Coulibaly *et al.* (2008a, 2008b) and Ouattara *et al.* (2009) (Fig.1). Each bed was filled from bottom to the surface with 0.1 m gravel (5/15 mm) covered with cloth and 0.6 m white lagoon sand, previously washed to remove any clay, loam and organic matter. The bed bottom slope was 1 % oriented via PVC of 0.032 m diameter to drain out the effluent of the bed. Each bed was equipped with irrigation devices consisted of 6 PVC pipe (length: 1.70 m; diameter; 0.008 m) containing 60 lateral holes.

Insert Fig. 1

Five plants namely *Andropogon gayanus* (Kunth, 1833), *Chrysopogon zizanioides* (Roberty, 1960), *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase (1917), *Pennisetum purpureum* (Schumach. 1827) and *Tripsacum laxum* (Nash, 1909) were experimented. These are perennial forage plants highly appreciated by agro-pastoralists for their palatability, adaptation to local climatic conditions and presence in Côte d'Ivoire (Boudet, 1991).

In order to minimize the variability in the experiment and address CWs clogging problems reported by Coulibaly *et al.* (2008a, 2008b) and Ouattara *et al.* (2008), synthetic domestic wastewater was employed in the experiment as the influent of the wetlands. The composition of synthetic domestic wastewater was made according Rodgers *et al.* (2006) and Healy *et al.* (2010). However, some modifications were done in order to respect the characteristics (*i.e.* nitrogen, phosphorus and carbon concentrations) of domestic wastewater encountered in developing countries according to Metcalf & Eddy (1991). The characteristics of the synthetic domestic wastewater used were: COD = 628 mg O₂/L, BOD₅ = 380 mg O₂/L, TN = 45 mg/L, TSS = 300 mg/L, TP = 12 mg/L, pH = 6.7- 8.00.

Experimental procedure:-

The effect of the five plants specie on the performance and macroinvertebrates biodiversity of vertical-flow CWs was examined over a seven-month period of experimentation. Five beds were transplanted with the plant seedlings (*i.e.* 9 plants/m²) spaced of 40 cm x 40 cm between the stems on monoculture and the sixth remained an unplanted control. These young plants were collected from nurseries established near the experimental pilot and previously cut to 20 cm above the roots before the bed planting.

The plants thus transplanted were fed with tap water for one month to allow acclimatize. After the acclimation period, each bed was intermittently fed (3 days/week) with 23.64×10^{-3} m³/d hydraulic loading of synthetic domestic wastewater according to Ouattara *et al.* (2008, 2009, 2011) over 6 months. The synthetic domestic wastewater tank was cleaned before and after the feeding of beds to remove all the impurities settled.

Wastewater samples were taken once a week at inlet (influent) and outlet (effluent) of each bed, stored in an ethylene bottle at 4 °C until analysis. A total of 24 water samples were taken in each bed during the whole period of the experiment. At the end of each two-month growth cycle, plants were harvested according to Ouattara *et al.* (2008) and the plant aboveground biomass produced was determined by weighing. Three measures of plant aboveground biomass were carried out during the experiment in each bed. For the macroinvertebrates study, sampling consisted of a collection of beds sediments at the end of the treatment trial.

Physical and chemical analyses and removal efficiency:-

For each water sample collected, pH, and dissolved oxygen (DO) were determined according to ISO 10523 (2008) and ISO 5814 (2012), respectively, while total suspended solids (TSS) by ISO 11923 (1997). Then, chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP) analyses were done according to ISO 6060/2 (1989), ISO 5663 (1984) and ISO 6878 (2004), respectively. Finally, removal efficiencies was calculated according to Abissy and Mandi (1999) for TSS, COD, TN and TP as follows:

$$\text{Removal efficiency (\%)} = \frac{C_i V_i - C_e V_e}{C_i V_i} \times 100 \quad (1)$$

Where C_i and C_e are the inlet and outlet concentrations (mg/L), V_i and V_e are the inlet and outlet volume (L) in the CWs.

Macrofauna sampling and identification:-

The characterization of macroinvertebrates communities was performed in six sediment layers in the vertical profile, from upper surface to the bottom of the bed: [0;-10 cm], [-10;-20 cm], [-20;-30 cm], [-30;-40 cm], [-40;-50 cm] and [-50;-60 cm] according to Environment Agency (2000) that was modified by Ouattara *et al.* (2009). This consisted of drawing quadrats of 25 cm² in the beds. Five quadrats (*i.e.*, one at each corner and one in the center of the beds) were selected for sampling macroinvertebrates. Thus, five sediment (10 cm × 10 cm × 10 cm) cores were taken from the five quadrats of each layer considered at the end of treatment trial. The five sediment cores of each layer were mixed to make the composite core of this one, stored in jars, preserved in 5% formaldehyde and analyzed in the laboratory.

Analysis consisted to pass under a water tap, the contents of each jar previously put in a sieve of 1 mm mesh vacuum, to leach out the sediments and eliminate formaldehyde. Sieve rejects were then recovered and organisms were sorted by hand magnifying glass and forceps under a light source and stored in pill containers containing 5% formaldehyde. Using a binocular loupe, these organisms were identified, counted and pooled by classes, orders and family, combining the identification keys of Bouché (1972) and Dejoux *et al.* (1981).

Data analysis:-

Pollutant and above-ground biomass data were not normally distributed (Shapiro-Wilk normality test), so the statistical tests used for data set analysis included the Kruskal-Wallis and Mann-Mann tests. Whitney. In this case, the Mann-Whitney test was used to compare the physico-chemical parameter values and the biomass of the plants between the beds two by two. The average values of physico-chemical parameters were used for the presentation of the results.

As for macroinvertebrates, several indices were used to analyze their settlement. The taxonomic richness (R) was simply taken as a count of number of taxa present in each bed. The diversity indices provide more information about community composition, about scarcity and commonness of taxa in a given community; Shannon-Wiener index (H), Equitability index (E) are commonly used to characterize taxa diversity in a community (Magurran, 1988). Shannon-Wiener diversity index was expressed following the equation (2) below, where NS is the proportion of individuals found in the taxon; NT is the number of taxa in the samples.

$$H = \sum \left(\frac{NS}{NT} \right) \log_2 \left(\frac{NS}{NT} \right) \quad (2)$$

Species equitability or evenness (E) was determined by the equation (3), where H was the Shannon-Wiener diversity index, R was the number of taxa in samples.

$$E = \frac{H}{\log_2 R} \quad (3)$$

The density is an important tool to measure macroinvertebrates (Barbour *et al.*, 1996). Thus, in this study, the relative abundance (Ra) estimated according to equation (4) below, was considered:

$$Ra \left(\frac{Ind.}{m^3} \right) = \frac{\text{Total number of taxa}}{\text{volume of sampling unit}} \quad (4)$$

Analysis of these indices were performed using Kruskal-Wallis and Mann-Whitney tests for taxa richness (R) and density (D) while ANOVA variances, and T-test were used for Shannon-Wiener Diversity (H) and equitability (E) to investigate differences between CWs bed. The statistical tests used were performed using R studio 3.3.2 software (Ihaka & Gentleman, 1996). In all tests, the differences were considered statistically significant for $p < 0.05$.

Results:-

Plant biomass production and wastewater treatment in CWs:-

Plant biomass production:-

Figure 2 illustrates the variation in plant biomass produced on the different beds of CWs during plants harvests during the treatment trial. The plant biomass ranged from 14.55 to 15.86 kg/m², 10.76 to 15.52 kg/m², 3.59 to 12.76 kg/m², 1.45 to 4.67 kg/m², 1.45 to 2.58 kg/m² on the beds transplanted with *P. purpureum*, *T. laxum*, *E. pyramidalis*, *A. gayanus* and *C. zizanioides*, respectively. Overall, the biomasses produced on the beds planted with *E. pyramidalis*, *P. purpureum* and *T. laxum* were significantly higher than those of *A. gayanus* and *C. zizanioides* (Mann Whitney test: $p < 0.05$). However, biomass produced on the bed transplanted with *P. purpureum* achieved the

highest above ground biomass and was followed by those of *T. laxum*, *E. pyramidalis*, *A. gayanus* and *C. zizanioides*, respectively.

Insert Fig. 2

Wastewater treatment:-

Table 1 exhibits the average results of the tested parameters and the pollutants removal efficiency (*i.e.*, TSS, COD, TN and TP) of the different beds (planted and unplanted).

We observe that the pH of influent increased from 6.81 to values between 6.92 and 7.32 in beds effluents. The sequence of pH mean values was: Influent (6.81) < effluent of B_{AG} (6.92) < effluent of B_{EP} (6.93) < effluent of B_{CZ} (7.05) < effluent of B_{PP} (7.06) < effluent of B_{TL} (7.17) < effluent of UB (7.32). Besides, significant differences were observed between wastewater pH and those of planted beds, and also among them obtained in the different beds (Mann Whitney test: $p < 0.05$). Also, the DO values increased in all beds effluents from 2.13 ± 0.55 mg/L in the influent to values ranging from 5.41 to 7.53 mg/L. However, the DO values of the effluents of the planted beds were greater than those of the unplanted bed (Mann Whitney test: $p < 0.05$). However, some significant differences were noted among those of the planted beds effluents (Mann Whitney test: $p < 0.05$).

TSS, COD, TN and TP of wastewater were significantly removed in the beds effluents (Mann Whitney test: $p < 0.05$). However, except TSS, the other pollutants were significantly more reduced in the planted beds, than in the control. Indeed, the influents concentrations of these parameters decreased from 284.9 ± 11.67 to 16.66 ± 1.29 mg/L, from 611.8 ± 18.02 to 36.67 ± 3.78 mg O₂/L, from 41.45 ± 2.24 to 9.60 ± 5.22 mg/L, and from 10.93 ± 0.49 to 0.86 ± 0.31 mg/L in the beds effluents, respectively for TSS, COD, TN and TP.

Considering the planted beds, the overall order of performance is as follows: performance of B_{PP} > performance of B_{TL} > performance of B_{EP} > performance of B_{AG} > performance of B_{CZ}. However, some significant differences were found between the beds performances (Kruskal-Wallis test, $p < 0.05$). However, the beds planted with *P. purpureum* and *T. laxum* were significantly efficient than those planted with *E. pyramidalis*, *A. gayanus* and *C. zizanioides* (Whitney test: $p < 0.05$).

Insert Table 1

Macroinvertebrates communities in CWs:-

Qualitative analysis of macroinvertebrates communities:-

Taxonomic composition and richness:-

Table 2 summarizes macroinvertebrates taxa collected in the beds sediment at the end of the treatment trial. Altogether, 12 taxa of macroinvertebrates were identified, belonging to 6 classes (*i.e.*, Acheta, Arachnida, Myriapoda, Insecta, Malacostraca and Oligochaeta), 12 orders (*i.e.*, Rhynelobdoliiforme, Araneae, Diplopoda, Chilopoda, Coleoptera, Diptera, Ephemeroptera, Hymenoptera, Lepidoptera, Orthoptera, Isopoda and Lumbricinas) and 12 families. The most diversified class was Insects, which represented 50% of the orders collected (Diptera, Coleoptera, Ephemeroptera, Hymenoptera, Lepidoptera and Orthoptera). It was followed by those of Myriapoda (Chilopoda and Diplopoda), the proportion of which was 16.7%. The other classes consist of one order each and each represented 8.3%.

Of the 12 taxa collected in the beds sediments, all were found in the beds transplanted with *T. Laxum* and *P. Purpureum*, 11 taxa were obtained in the beds transplanted with *E. Pyramidalis* and *A. Gayanus*, 9 in the bed transplanted with *C. Zizanioides* and 7 in the unplanted bed. However, all the beds had in common 3 classes (Insecta, Malacostraceae and Oligochaeta) divided into 7 orders (Coleoptera, Diptera, Ephemeroptera, Hymenoptera, Lepidoptera, Isopoda and Lumbricinas) and 7 families (Passalidae, Chironomidae, Ephemerellidae, Formicidae, Crambidae, Aselidae and Lumbricidae).

Considering the planted beds, they had in common 9 taxa. Arachnidae were found only in the beds transplanted with *T. Laxum* and *P. Purpureum* while, Glossiphoniidae and Acrididae were absent in the bed transplanted with *C. Zizanioides*. The number of taxa encountered in the beds sediments differed significantly between planted and unplanted beds (Mann Whitney test, $p < 0.05$). However, between the planted reactors, it remains similar ($p > 0.05$).

Insert Table 2**Diversity of macroinvertebrates settlements:-**

Figure 3 illustrates the variations in the Shannon diversity index and the Equitability of macroinvertebrates living in the sediment of CWs beds. It appeared that macroinvertebrates settlements in planted beds had higher index values than those recorded in the control.

For the Shannon diversity index (Fig. 3A), the values varied from 1.93 to 2.19 bits/ind. in the bed planted with *T. Laxum*, from 1.84 to 2.16 bits/ind. in that planted with *A. Gayanus*, from 1.53 to 1.95 bits/ind. in the bed planted with *C. Zizanioides*, from 1.84 to 2.12 bit/ind. in the bed planted with *E. Pyramidalis*, from 2.09 to 2.28 bits/ind. In the bed planted with *P. Purpureum* and from 1.24 to 1.71 bits/ind. in unplanted bed. The corresponding average values remained high in the bed planted with *P. Purpureum* (2.19 bits/ind.) and was Followed by those the beds planted with *T. Laxum* (2.06 bits/ind.), *A. Gayanus*, (2.01 bits/ind.), *E. Pyramidalis* (2.00 bits/ind.), *C. Zizanioides* (1.74 bits/ind.) and the control (1.47 bits/ind.), respectively. The statistical analysis revealed a significant difference between the index values of the control and those of the planted beds except that planted with *C. Zizanioides* (T test, $p < 0.05$). Considering the planted beds, only the index values recorded in the beds planted with *P. Purpureum* and *C. Zizanioides* differed statistically (T test, $p < 0.05$).

As for the equitability index (See Fig. 3B), it oscillated between 0.78 and 0.88, between 0.77 and 0.9, between 0.69 and 0.89, between 0.77 and 0.88 between 0.84 and 0.92 and between 0.64 and 0.88 respectively in the beds planted with *T. Laxum*, *A. Gayanus*, *C. Zizanioides*, *E. Pyramidalis*, *P. Purpureum* and the control. The comparison of the average equitability values recorded in the planted beds showed that, that of the bed planted with *P. Purpureum* was the highest while that of bed planted with *C. Zizanioides* was the weakest. However, no significant difference was noted between the equitability values of the different beds (ANOVA test, $p > 0.05$).

Insert Fig. 3**Quantitative analysis of macroinvertebrates communities:-****Abundances of macroinvertebrates settlements:-**

A total of 600 individuals of macroinvertebrates were collected in the beds sediments of the CWs (Table 3). This table showed that, the beds transplanted had a greater number of macroinvertebrates (between 50-205 individuals, i.e. $1.67 \cdot 10^3$ - $6.83 \cdot 10^3$ Ind./m³) than, the unplanted bed (37 individuals, corresponding to $1.23 \cdot 10^3$ Ind./m³) (Mann-Whitney test: $p < 0.05$). However, the bed planted with *P. Purpureum* recorded the highest number of macroinvertebrates of 205 individuals (i.e. $6.83 \cdot 10^3$ Ind./m³). It was followed by those planted with *T. Laxum* (132 individuals, i.e. $4.40 \cdot 10^3$ Ind./m³), *E. Pyramidalis* (101 individuals, i.e. $3.38 \cdot 10^3$ Ind./m³), *A. Gayanus* (75 individuals, i.e. $2.50 \cdot 10^3$ ind / m³) and *C. Zizanioides* (50 individuals, i.e. $1.67 \cdot 10^3$ Ind./m³), respectively.

Insert Table 3

Examination of the macroinvertebrates settlements in the beds sediments indicated on the whole that the families (i.e. Formicidae and Lumbricidae) were the most abundant. The number of these organisms was respectively 7 ($0.23 \cdot 10^3$ Ind./m³) and 19 ($0.63 \cdot 10^3$ Ind./m³) in unplanted bed, 31 ($1.03 \cdot 10^3$ Ind./m³) and 30 (10^3 Ind./m³) in the bed planted with *T. Laxum*, 14 ($0.47 \cdot 10^3$ Ind./m³) and 23 ($0.77 \cdot 10^3$ Ind./m³) in the bed planted with *A. Gayanus*, 10 ($0.33 \cdot 10^3$ Ind./m³) and 21 ($0.70 \cdot 10^3$ Ind./m³) in the bed planted with *C. Zizanioides*, 24 ($0.80 \cdot 10^3$ Ind./m³) and 25 ($0.83 \cdot 10^3$ Ind./m³) in the bed planted with *E. Pyramidalis*, and 44 ($1.47 \cdot 10^3$ Ind./m³) and 38 ($1.27 \cdot 10^3$ Ind./m³) in the bed planted with *P. Purpureum*.

Depending on the classes of macroinvertebrates collected, we noted that insecta and oligochaeta appeared the most abundant (Fig. 4). These organisms occupied respective proportions of 43.9 and 22.7% in the bed planted with *T. Laxum*, 41.3 and 30.7% in the bed planted with *A. Gayanus*, 42 and 42% in the bed planted with *C. Zizanioides*, 44.6 and 24.8% in the bed planted with *E. Pyramidalis*, 47.8 and 18.5% in the bed planted with *P. Purpureum*, and 40.5 and 51.4% in the unplanted bed. However, Insects dominated all the planted beds, while Oligochaeta were the most abundant in unplanted bed.

Insert Fig. 4**Vertical distribution of macroinvertebrates settlements:-**

Table 4 exhibits the vertical distribution of the number of macroinvertebrates recorded in the beds sediment layers. Overall, the number of individuals of macroinvertebrates decreased from the upper layer (0-10 cm) to the bottom layer (50-60 cm) of the beds. This varied from 63 to 1 (i.e. $12.6 \cdot 10^3$ - $0.2 \cdot 10^3$ Ind./m³), from 38 to 2 (i. e, $7.6 \cdot 10^3$ - 0.4

10^3 Ind./m³), from 26 to 2 (i.e. $5.2 \cdot 10^3$ - $0.4 \cdot 10^3$ Ind./m³), from 48 to 2 (i.e. $9.6 \cdot 10^3$ - $0.4 \cdot 10^3$ Ind./m³), from 96 to 1 (i.e. $19.2 \cdot 10^3$ - $0.2 \cdot 10^3$ Ind./m³) and from 16 to 2 (i.e. $3.2 \cdot 10^3$ - $0.4 \cdot 10^3$ Ind./m³) individuals, respectively in the beds planted with *T. laxum*, *A. gayanus*, *C. zizanioides*, *E. pyramidalis*, *P. purpureum* and in the unplanted bed. Except the two last bottom layers (40-50 cm and 50-60 cm), number of individuals collected in the sediments of the planted beds was higher than that obtained in the control. Comparing the number of macroinvertebrates recorded in each of the layers of the planted beds, it appeared that, that of the bed planted with *P. Purpureum* remained higher. It was followed by those of the beds planted with *T. Laxum*, *E. Pyramidalis*, *A. Gayanus* and *C. Zizanioides*, respectively. In the two last layers of the bottom, it was noted that the beds planted with *P. Purpureum* and *T. Laxum* were the least populated.

Insert Table 4

Discussion:-

Plant biomass production:-

The different macrophytes (i.e., *P. purpureum*, *T. laxum*, *E. pyramidalis*, *A. gayanus* and *C. zizanioides*) experimented, showed good growth for having developed large above ground biomasses ranging from 14.55 to 15.86 kg/m², from 10.76 to 15.52 kg/m², from 3.59 to 12.76 kg/m², from 1.45 to 4.67 kg/m², and from 1.45 to 2.58 kg/m², respectively in the CWs. The production of such biomasses in the CW could be explained by the nutrients content (i.e., nitrogen and phosphorus) and organic matter of the applied wastewater and by the climatic conditions of the study area (tropical climate) (Ouattara *et al.*, 2008).

Overall, these biomasses were of the same order of magnitude as estimated by Talineau (1968) and Sefiétiou *et al.* (2005), who obtained in the wild mean fresh plant biomass of 20.0 kg/m² of *P. purpureum*, 12.0 kg/m² of *T. laxum*, 12.0 kg/m² of *E. pyramidalis*, 3.1 kg/m² of *C. zizanioides* and 3.0 kg/m² of *A. gayanus*. However, *P. purpureum* appeared to be the best adapted plant species to the culture medium for having achieved the highest above ground biomass during the experience. In fact, this plant species has long been investigated as a species that provides a significantly age-related yield of dry matter that is adapted to the tropics and can be grown and harvested all year round for agro-industrial purposes (Ferraris & Sinclair, 1980). Pillai and Vijayan, (2013) even obtained higher biomasses of 20-25 kg/m² in a vertical-flow CW, but using a crop association of *Pennisetum* varieties (i.e. *P. purpureum* and *P. typhoides*).

CWs performance:-

Monitoring of physico-chemical parameters of raw water (influent) and those of the filtrates (effluents) of the beds during the treatment trial revealed higher values of pH and DO in the filtrates compared to raw water. In addition, unlike DO, the pH of the control effluent appeared greater than those of the planted beds. The increase of pH values in the effluents could be explained by the activity of denitrifying bacteria in the deep layers of the developed vertical flow CWs. The work of Finlayson and Chick (1983) and Koné *et al.* (2011) also report increasing pH in the planted beds effluents. In addition, the highest pH values observed in the control filtrate compared with those of planted beds could be due to the action of plants (Shelef *et al.*, 2013). The increase in DO in the effluents would result from the aeration of the untreated water during its application to the vertical flow CW beds used in this study. In addition, small amounts of oxygen from the aerial parts are rejected at the apex of rootlets of plants that could contribute to higher oxygen levels in the influents of planted beds (Poulet *et al.*, 2004; Pérez *et al.*, 2014). However, the different noted between beds effluent DO would be due to a likely variation in the amount of oxygen released into the plant's rhizosphere in the CW, depending on the plant's physiology (Stottmeister *et al.*, 2003; Gagnon *et al.*, 2007).

Concerning pollutants (e. g. TSS, COD, TN and TP), all were significantly removed in the beds effluents. However, except TSS, the other pollutants were significantly more reduced in the planted beds, than in the control. The significant reduction of pollutants in all the bed effluents could be due to CW removal process (i.e., macrophytes assimilation, precipitation, dissolution, adsorption or desorption), but especially, to sediment storage and microbial uptake (Yang *et al.*, 2001, Vymazal, 2006, Ruiz-Rueda *et al.*, 2009). Indeed, microbial processes and sediment storage on the performance of wetland treatment have been widely documented and considered as determinant removal pathways in CWs (Faulwetter *et al.*, 2009; Wu *et al.* 2013a, 2013b). Thus these reduction would be linked on the one hand, to the white lagoon sand used in this study (having strongly retained suspended solids) and a good colonization of the beds sediments by the purifying microorganisms and, on the other hand, to a good oxygenation of the sediment during the rest phase as observed Molle *et al.* (2005), Ouattara *et al.* (2008) and Kone *et al.* (2011). In fact, the rest phases of the beds favor a greater recharge of the bulk of oxygen useful for the metabolism of the bacteria during the biodegradation of the pollutants.

However, many studies like those of Molle *et al* (2006), Coulibaly *et al.* (2008a, 2008b) and Ouattara *et al.* (2008), reported that plants would developed channels via roots in the beds sediment, which should improve planted beds permeabilities, contributing in this way to water infiltration into them, than in the control. These channels would favored the transit of suspended solids in the effluent of the planted beds, unlike control's that remained devoid of plants, justifying the strong reduction of TSS in control filtrates compared to those of planted beds. However, the best TSS reduction noted among the effluents in the bed planted with *P. purpureum* could be attributed to the root system of this species. Indeed, unlike other species, it was found during the demolition of the beds at the end of the treatment trial that the roots of *P. purpureum* had formed a kind of mat much denser than the other plants at the bottom of the bed. This would have behaved as a second filter at the bottom of the bed which would have decreased more the TSS in this bed. However, if the results of this study were in disagreement with that of Pillai and Vijayan (2013), who obtained the lowest TSS removal rate (65%) in the control against 89% and 93%, respectively in the beds planted with *P. maximum* and a mosaic of (*P. purpureum* + *P. typhoides*), this should be justified by the use of gravel, sand and coconut fibers as filtration materials.

On the other hand, the greatest removing of DOC, TN and TP recorded in planted beds than, in the control would be related to the presence of the plants species. Indeed, according to several reviews of the ways in which plants can affect CW processes, such as those for Brix, (1997), Vymazal, (2011) and Shelef *et al.* (2013), to name but a few, plants would confer more favorable ecological conditions, improving removal process unlike the unplanted bed. In addition to the assimilation of nutrients for their growth, plants create within the sediment, an aerobic micro-habitats (in the upper layer) and anaerobic (in the bottom layer) conducive to the growth of the purifying organisms (Brix, 1997; Vymazal, 2011). Moreover, plants constitute a source of carbon supply necessary for the renewal of the energy of the organisms through secreted exudates and maintain, thanks to the shading they provide, hygrometry essential to the good development of the fauna (Andrews & Harris, 2000, Karjalainen *et al.*, 2001; Vymazal, 2011). However, the overall performance of beds that were categorized as follows: Bed planted with *P. purpureum* > Bed planted with *T. laxum* > Bed planted with *E. pyramidalis* > Bed planted with *A. gayanus* > Bed planted with *C. zizanioides*, could be related, on the one hand, to the difference between plant biomass developed in this study, and on the other hand, to the secretion of root exudates which would vary from one plant species to another species (Stottmeister *et al.*, 2003; Vymazal, 2007). Thus, the highest biomasses developed by *P. purpureum* would have allowed a more abundant development of bacteria that would have made the contribution of this plant the best. However, the mean concentrations of TSS, COD, TN and TP in the beds effluent are below the limit values (50 mg TSS / L, 300 mg O₂ COD / L, 50 mg TN / L and 15 mg TP/ L) allowed in the regulation of wastewater discharges in Côte d'Ivoire (Ministry of Environment, Water and Forests, 2008).

Macroinvertebrates communities:-

The analysis of macroinvertebrates living in the CWs showed that the abundance and distribution of these organisms in the beds sediments were more or less influenced by the plant species studied. The presence of organisms in the CWs could be explained by various ecological factors, particularly the favorable physicochemical conditions and sufficient nutrient resources, to guarantee their survival (Gagnon *et al.*, 2007, Ouattara *et al.*, 2009; 2011).

The inventory of the macroinvertebrates in the beds of the CWs has allowed identifying 12 families belonging to 6 classes, of which that of the Insects appeared the most diversified. This situation should due to the large taxocenosis of the Insect class (Tachet *et al.*, 2003). However, in addition to the classes of macroinvertebrates recorded by Ouattara *et al.* (2009; 2011), one new class (*i.e.* Acheta) was found in the bed sediment in this study. In fact, Acheta are pollution resistant taxa often parasitic of invertebrates, of the large faunistic group of Annelids, proliferating favorably in humid environments of high degree of organic pollution (Abbou & Fahde, 2014). Thus, these organisms would have been transported on the reactors by higher animals, attracted by the plants, and would have favorably swarmed the planted reactors, because of the strong vegetation cover of the latter.

The Shannon diversity index values (1.74-2.19 bits/ind.) and Equitability (0.64-0.90 bits/ind.) obtained in this study indicated that the macroinvertebrates settlements recorded in the CWs beds sediments were little diversified and poorly organized (Dajoz, 2000). This diversity of macroinvertebrates settlements in the CWs could be explained by the duration of this treatment trial. The present study, which lasted 6 months, would be relatively short to allow for significant bed colonization. However, analysis of Shannon diversity index values, Equitability and the abundance of taxa indicated that macroinvertebrates were more diversified and more abundant in planted beds than in the control. This situation confirmed the assumed role of plants in generating micro aerobic environment in the rhizosphere and a hygrometry favorable to the colonization of planted beds thanks to the shading they provide (Brix,

1997, Andrews & Harris 2000, Karjalainen *et al.*, 2001, Wießner *et al.*, 2002). This result corroborated those of Gagnon *et al.* (2007) and Ouattara *et al.* (2009). However, the results indicated that between the planted beds, that planted with *P. Purpureum* was the most diversified and had more taxon. It was followed by beds planted with *T. Laxum*, *E. Pyramidalis*, *A. Gayanus* and *C. Zizanioides*, respectively. The settlement difference observed between the planted beds could likely be related to the difference between plant biomass abundance of the tested plants species, especially since the order of abundance of the taxa of the planted beds was similar to that of the abundance of plant biomass produced.

The quantitative analysis of the macroinvertebrates settlement showed that the macroinvertebrates abundance was significantly higher in the planted beds than in the control. This result could be justified by the presence of the different plants, which would have conferred more favorable ecological conditions for proliferation of the fauna. Ouattara *et al.* (2009; 2011) recorded also greater number of macroinvertebrates settlement in beds planted than in the control. On the other hand, the proportion of insects ranging from 40.5 to 47.8% and that of Oligochaeta fluctuating between 22.7 and 51.4% dominated the macroinvertebrates settlement recorded in CW bed sediments. According to Mason (1991), the abundance of these two classes of macroinvertebrates could be explained by the fact that they contain opportunistic taxa, which are able to monopolize the available resources, allowing them to survive longer than the others. However, the result showed that insects dominated most of the planted reactors while Oligochaeta dominated the settlement in the control. The abundance of Oligochaeta in the sediment of the control could be explained by the fact that these organisms being scavengers, they would take advantage of the significant accumulation of the organic matter from the wastewater in this bed one compared to the planted beds, to pullulate (Berry & Jordan, 2001). As for the dominance of Insects in planted beds, this would be justified by their diet. Indeed, according to Gobat *et al.* (1998) and Lamarre *et al.* (2012), Coleoptera, Diptera, Ephemeroptera, Lepidoptera, abundantly found in planted beds, are omnivorous and scavenger. As a result, these organisms would have been attracted to plants, biofilm, organic debris and microscopic algae contained in the planted beds.

Results showed a decreasing of the abundance of macroinvertebrates with depth, following the vertical profile in all the CWs beds sediment. These results were similar to those obtained by Ouattara *et al.* (2009; 2011), investigating the effect of *P. maximum* on macrofauna communities in a CW. These authors attributed this situation to the living conditions of this environment, where oxygen and available energy resources would decrease with depth. Indeed, several studies report the decrease in oxygen concentration and the amount of nutrients required for optimal growth of organisms (Münch *et al.*, 2005, Truu *et al.*, 2009). These would decrease significantly from the first ten centimeters and become rare beyond 20 cm deep.

Conclusion:-

This study confirmed the contribution of plants to the wastewater treatment process in constructed wetlands. All the five experimented plant species grew well and their presence improved significantly pollutants removal into the beds even if TSS removal in the control was slightly higher than those in the planted beds. Average concentrations of TSS, COD, TN and TP in the effluents of all planted beds were below the current standards of wastewater discharges in Cote d'Ivoire of 50 mg TSS/L, 300 mg O₂ COD/L, 50 mg TN/L and 15 mg TP/L. However, the plant species, *P. purpureum* is suggested in constructed wetlands for the treatment of domestic wastewater, for having contributed the most to the removal of pollutants from wastewater.

Overall macroinvertebrates settlement within the beds was little diversified. Twelve families belonging to 6 classes were recorded with one new class (*i.e.*, Acheta) in addition to the classes of macroinvertebrates previously recorded. The presence of the different plant species had a significant impact on macroinvertebrates diversity and their abundance. Indeed macroinvertebrates were more diversified and more abundant in the planted beds than in the control. Insects dominated all the planted beds, while Oligochaeta were the most abundant in unplanted bed. However, the bed planted with *P. purpureum* housed the largest number of organisms and was followed by those planted with *T. laxum*, *E. pyramidalis*, *A. gayanus* and *C. zizanioides*, respectively. Following the vertical profile, macroinvertebrates settlement abundance decreased from the upper layer (0-10 cm) to the bottom layers (50-60 cm).

This research indicates that the study of macroinvertebrates is adequate when comparing the effect of plant species, inasmuch as the most appropriate plant has favored the greatest proliferation of organism. Further studies should be carried out to assess the influence of plants presence and species in full size to validate the finding obtained in this experience in pilot-scale.

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Table 1:- Mean average and standard deviations of physico-chemical parameters inlet (influent) and outlet (effluents) the beds and average removal Influent and effluent, B_{TL} = bed planted with *T. laxum*, B_{AG} = bed planted with *A. gayanus*, B_{CZ} = bed planted with *C. Zizanioides*, B_{EP} = bed planted with *E. Pyramidalis*, B_{PP} = bed planted with *P. purpureum*, UB = unplanted bed and NA= not applicable

	TSS (mg/L)	Removal (%)	COD (mg O ₂ /L)	Removal (%)	TN (mg/L)	Removal (%)	TP (mg/L)	Removal (%)	pH	DO (mg/L)
Influent	284.9± 11.67	NA	611.8± 18.02	NA	41.45± 2.24	NA	10.93± 0.49	NA	6.81± 0.07 ^a	2.13± 0.55 ^a
Effluents										
B_{TL}	28.55± 2.92	93.00± 1.05 ^a	48.70± 12.07	94.46± 1.39 ^{ab}	10.98± 4.23	81.59± 08.41 ^a	1.53± 0.41	90.26± 2.90 ^a	7.17± 0.30 ^{cb}	7.53± 1.56 ^c
B_{AG}	34.37± 6.19	90.86± 1.91 ^b	73.90± 15.89	90.91± 1.89 ^{ab}	12.83± 5.38	76.66± 11.16 ^b	2.61± 1.09	81.97± 8.01 ^b	6.92± 0.26 ^a	6.50± 0.80 ^d
B_{CZ}	39.48± 5.91	89.20± 1.82 ^c	63.37± 5.58	91.90± 0.90 ^a	13.59± 4.02	74.48± 10.00 ^b	3.71± 0.66	73.56± 5.01 ^b	7.05± 0.27 ^c	6.70± 1.04 ^d
B_{EP}	29.53± 3.09	92.30± 1.42 ^a	55.03± 7.17	93.38± 1.08 ^{ab}	13.28± 5.15	76.54± 11.67 ^b	1.99± 1.14	86.64± 8.39 ^b	6.93± 0.27 ^a	6.63± 0.98 ^d
B_{PP}	25.98± 1.92	93.81± 0.80 ^b	36.67± 3.78	95.92± 0.68 ^b	09.60± 5.22	84.30± 09.56 ^a	0.86± 0.31	94.68± 2.10 ^a	7.06± 0.21 ^c	7.24± 1.06 ^c
UB	16.66± 1.29	94.70± 0.45 ^c	150.2± 31.87	77.70± 5.03 ^c	19.40± 5.27	57.66± 14.98	6.62± 0.34	45.24± 3.91	7.32± 0.30 ^b	5.41± 0.88 ^b

Values within the same column followed by the same superscript letter (*i.e.* a, b, c ...) are not significantly different at $p < 0.05$

Table 2:- List of macroinvertebrates communities recorded in the beds sediment, B_{TL} = bed planted with *T. laxum*, B_{AG} = bed planted with *A. gayanus*, B_{CZ} = bed planted with *C. Zizanioides*, B_{EP} = bed planted with *E. Pyramidalis*, B_{PP} = bed planted with *P. purpureum*, UB = unplanted bed (* = presence of the considered taxon)

Macroinvertebrates			Beds						
Classes	Orders	Families	B _{TL}	B _{AG}	B _{CZ}	B _{EP}	B _{PP}	UB	
Acheta	Rhynelobdoliforme	Glossiphoniidae	*	*		*	*		
Arachnida	Aranea	Arachnidae	*				*		
Insecta	Coleoptera	Passalidae	*	*	*	*	*	*	
	Diptera	Chironomidae	*	*	*	*	*	*	
	Ephemeroptera	Ephemerellidae	*	*	*	*	*	*	
	Hyménoptera	Formicidae	*	*	*	*	*	*	
	Lepidoptera	Crambidae	*	*	*	*	*	*	
	Orthoptera	Acrididae	*	*		*	*		
Malacostraca	Isopoda	Aselidae	*	*	*	*	*	*	
Myriapoda	Chilopoda		*	*	*	*	*		
	Diplopoda		*	*	*	*	*		
Oligocheta	Lumbricina	Lumbricidae	*	*	*	*	*	*	
6	12	12	12	11	9	11	12	7	
Taxa in common to planted beds			9						
Taxa in common to all beds			7						

Table 3:-Abundance of macroinvertebrates settlements collected in the CW sediments, B_{TL} = bed planted with *T. laxum*, B_{AG} = bed planted with *A. gayanus*, B_{CZ} = bed planted with *C. Zizanioides*, B_{EP} = bed planted with *E. Pyramidalis*, B_{PP} = bed planted with *P. purpureum*, UB = unplanted bed

Macroinvertebres			Beds					
Classes	Orders	Families	B_{TL}	B_{AG}	B_{CZ}	B_{EP}	B_{PP}	UB
Acheta	Rhynelobdoliforme	Glossiphoniidae	6	2	0	3	11	0
Arachnida	Aranea	Arachnidae	2	0	0	0	4	0
Insecta	Coleoptera	Passalidae	11	7	4	8	20	4
	Diptera	Chironomidae	7	5	4	6	13	2
	Ephemeroptera	Ephemerellidae	3	2	2	2	8	1
	Hyménoptera	Formicidae	31	14	10	24	44	7
	Lepidoptera	Crambidae	5	2	1	4	8	1
	Orthoptera	Acrididae	1	1	0	1	5	0
Malacostraca	Isopoda	Aselidae	24	11	4	19	34	3
Myriapoda	Chilopoda		6	4	3	4	9	0
	Diplopoda		6	4	1	5	11	0
Oligocheta	Lumbricina	Lumbricidae	30	23	21	25	38	19
Number of macroinvertebrates in each bed			132	75	50	101	205	37
Total number of macroinvertebrates			600					

Table 4:-Vertical distribution of macroinvertebrates abundance in the CW sediments, B_{TL} = bed planted with *T. laxum*, B_{AG} = bed planted with *A. gayanus*, B_{CZ} = bed planted with *C. Zizanioides*, B_{EP} = bed planted with *E. Pyramidalis*, B_{PP} = bed planted with *P. purpureum*, UB = unplanted bed

	Abundances of macroinvertebrates					
	Beds					
Sediment layers (cm)	B_{TL}	B_{AG}	B_{CZ}	B_{EP}	B_{PP}	UB
0-10	63	38	26	48	96	16
10-20	36	22	13	28	57	10
20-30	17	7	5	12	31	4
30-40	10	4	2	8	13	3
40-50	5	2	2	3	7	2
50-60	1	2	2	2	1	2
Abundances in each bed	132	75	50	101	205	37
Total abundance	600					



Fig.1:- View of the experimental wetland system, 1: feeding tank, 2: bed, 3: Irrigation device and 4: plants growing on the beds after two months.

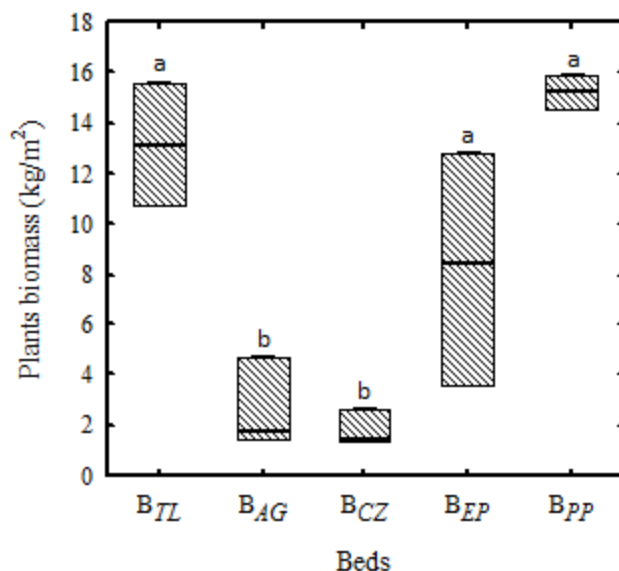


Fig.2:-Variation of aboveground plant biomass produced on the different beds in two months during three harvesting periods of the different studied plant species, B_{TL} = bed planted with *T. Laxum*, B_{AG} = bed planted with *A. Gayanus*, B_{CZ} = bed planted with *C. Zizanioides*; B_{EP} = bed planted with *E. Pyramidalis* and B_{PP} = bed planted with *P. Purpureum*

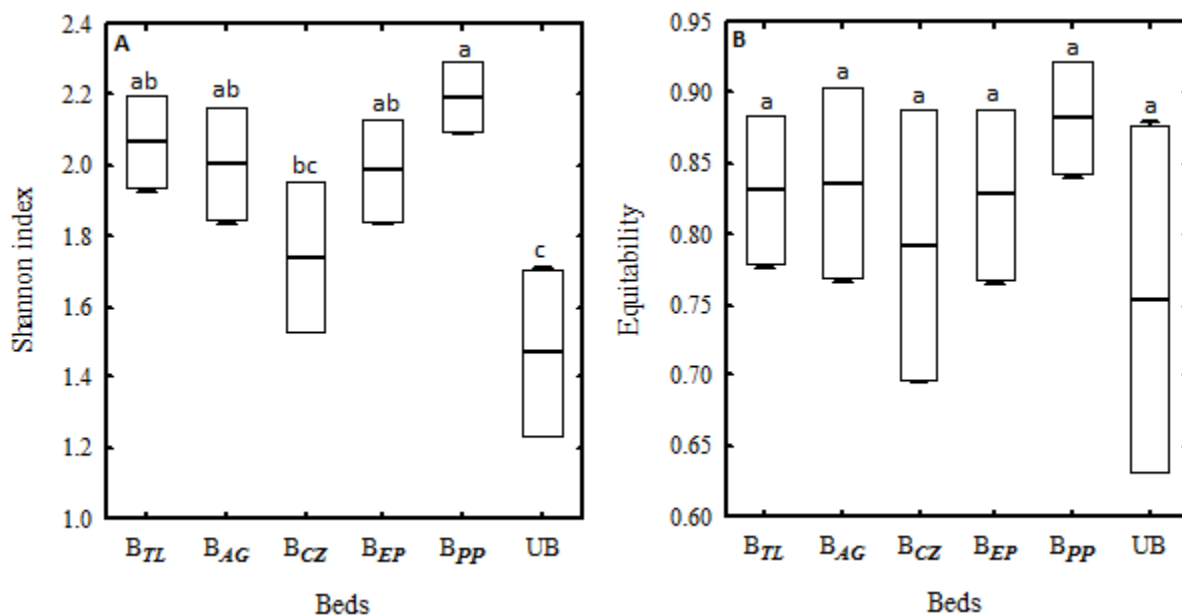


Fig.3:-Variation of Shannon index (A) and Equitability (B) in the beds of CWs, B_{TL} = bed planted with *T. laxum*, B_{AG} = bed planted with *A. gayanus*, B_{CZ} = bed planted with *C. Zizanioides*, B_{EP} = bed planted with *E. Pyramidalis*, B_{PP} = bed planted with *P. purpureum*, UB = unplanted bed, Box-plot marked with different letters differed significantly according to T test at $p < 0.05$.

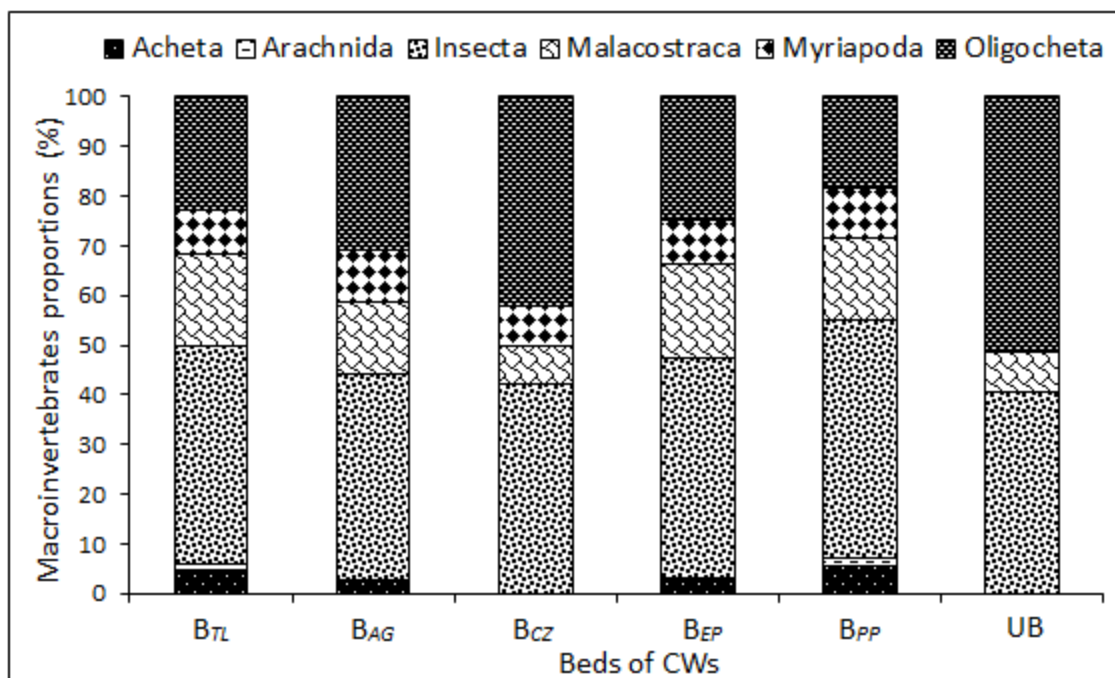


Fig.4:-Proportion of macroinvertebrates classes collected in the CW sediments, B_{TL} = bed planted with *T. laxum*, B_{AG} = bed planted with *A. gyanus*, B_{CZ} = bed planted with *C. Zizanioides*, B_{EP} = bed planted with *E. Pyramidalis*, B_{PP} = bed planted with *P. purpureum*, UB = unplanted bed

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