Bioremediation of Asa River Sediment Using Agricultural By-Products

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H. Sarma, S. Joshi (eds.), *Land Remediation and Management: Bioengineering Strategies*, https://doi.org/10.1007/978-981-99-4221-3_13

Abstract

The bioremediation of Asa River sediment using agricultural wastes such as rice husk and abattoir effluent was investigated through characterization of the river sediment and agricultural wastes with the aim of finding a solution to Ilorin's low soil fertility. The physicochemical characteristics of the sediment were evaluated. Using a serial dilution method, various fungi were isolated from the river sediment as well as the organic amendments. The isolated fungi were utilized for the bioremediation of the river sediment, and the biological activities (basal respiration, dehydrogenase activity, phytotoxicity, and microbial biomass) during the remediation process were studied. The ANOVA method was used to analyse the data obtained from the bioremediation experiments. The results showed that the sediment sample contains high concentrations of organic carbon, organic matter, and heavy metals, which were attributed to industrial wastes and agricultural run-off. Results also showed that higher amounts of the metals present in the river sediment were very phytotoxic ($p < 0.05$) and prevented crop germination. A total of 21 fungi were isolated from the sediment and agricultural wastes, and they significantly degraded the heavy metals in the sediment. Among the fungi, *Aspergillus niger* was the most effective in degrading most of the heavy metals, except for nickel, where *Fusarium solani* had the highest degradation. This study presents bioremediation as a low-cost and environmentally benign technique for remediating Asa River sediment.

Keywords

Abattoir effluent · Adsorption · Bioremediation · Heavy metal · Rice husk · River sediment

13.1 Introduction

For its considerable contribution to economic growth and human well-being, industrialization is considered the cornerstone of development initiatives (Iloamaeke and Iwuozor 2018; **Iwuozor et al.** 2021b; Kartam et al. 2004). Industrialization frequently results in pollution and degradation of the environment, just like many other anthropogenic activities that have an impact on the environment (Iwuozor 2019; Ogunfowora et al. 2021; Zhul-quarnain et al. 2018). Depending on the type of industry and the population that utilizes the product, industries produce waste that is unique in terms of composition and quantity. The continuous increase of industries

has resulted in a significant increase in the discharge of industrial waste into the environment, mostly soil and water, resulting in the deposition of various pollutants, particularly in metropolitan areas (Colombo et al. 2003; Iwuozor and Gold 2018).

Sediments, sometimes referred to as silt or alluvium, are made up of solid mineral and organic particles that are moved by water (Bruins et al. 2020; Yanin 2019). The sediment of a river serves as a sink for pollutants that enter the stream (Emenike et al. 2022; Pak et al. 2021). Both the transport capacity of the flow and the supply of sediment determine the quantity of sediment transported in river systems. The suspended sediment load refers to fine sediment transported in suspension, which might include material collected from the river bed (suspended bed material) as well as material washed into the river from the surrounding land (wash load). The suspended bed material is frequently finer than the wash load. The "bed load", on the other hand, consists of larger sediment particles carried along the river bed by rolling, sliding, or saltation. Depending on the flow conditions, most rivers will deliver silt in each of these load types (McKenzie et al. 2021; Sulaiman et al. 2021; Sumaiya et al. 2021).

Industrial pollution, overuse of water, ecosystem loss, and deterioration of aquatic habitats are all affecting river systems across the world (Haryani 2021; **Iwuozor et al**. 2021a; Marimuthu et al. 2020). As a result of pollution from atmospheric deposition, petrochemical spillage, coal combustion residues, wastewater irrigation, pesticides, sewage sludge, animal manures, land application of fertilizers, leaded gasoline and paints, disposal of high metal wastes, and rapidly expanding industrial areas, heavy metals and metalloids accumulate in dredged sediment (**Iwuozor** et al. 2021c; Ogemdi 2019a; Ogemdi 2019b; Ogunlalu et al. 2021). Sediment can also be useful or harmful to the society or the environment, depending on local variables. From an economic, social, and environmental standpoint, effective sediment management in rivers is becoming increasingly vital. The global geochemical cycle relies heavily on sediment delivered by rivers (Al Masud et al. 2018; Lin et al. 2020; Zhang et al. 2021). A large quantity of soil nutrients (nitrogen, phosphorus, and potassium) and cations are also present in river sediments, making the sediment beneficial for agronomy operations (Offiong et al. 2021; Yu et al. 2020). Bioremediation is commonly preferred for the remediation of soil or river sediments due to its low cost in comparison to other conventional techniques, its use as a permanent solution, its non-invasive nature, and its ability to clean up contaminants even at low concentrations, which chemical and physical techniques may not be able to do (Fragkou et al. 2021; Fu et al. 2020; Perelo 2010).

The Asa River runs south-north through Ilorin, splitting the plain into two parts: western and eastern. It is an important river in Ilorin, the capital city of Kwara State, with economic, environmental, and agricultural significance. The river covers around 303 hectares of land (Ighalo et al. 2021; Oladipo et al. 2020; Opasola et al. 2019). This river is very susceptible to pollution due to its proximity to industrial congestion, which exposes it to misuse such as effluent receptacles, which can lead to contamination. It collects waste from industries placed along its path on a regular basis, in addition to domestic waste and other activities that contribute to pollution. When portable water is not easily accessible, residents living along the river's

channel utilize the water for various domestic activities such as drinking, nursing wet vegetables, washing automobiles, and other household necessities (Ajala et al. 2018; Akinboro et al. 2021; Jimoh and Kolawole 2021). Local farmers use the sediment from the Asa River for agricultural cultivation in its existing form, without taking into account the level of contaminants present in the sediment. The bioremediation of the sediments could be implemented as a simple treatment to solve the problem of low soil fertility status in some areas of Ilorin. This presents an interesting novelty for the current investigation.

Some researchers have studied the characteristics and remediation of the sediment of the Asa River. Fawole et al. (2017) determined the physicochemical characteristics of the river sediment and also isolated six different fungal species from the river sediment. In different studies, the physicochemical properties of river sediment were also explored, and the heavy metals in the sediment were remediated with the use of abattoir effluent and poultry droppings (Adegbite et al. 2018; Augie et al. 2018). However, these studies were limited as they did not monitor the biological activities of the microbial isolates obtained from the wastes and utilized for the bioremediation process. The aim of this study was to bio-remediate Asa River sediment with the use of two agricultural by-products: rice husk and abattoir effluent. It involved the characterization of the river sediment together with the agricultural by-products, observation of biological activities (basal respiration, dehydrogenase activity, phytotoxicity, and microbial biomass) during the remediation process, and evaluation of the effects of individual fungal isolates on the detoxification of the river sediment. Given the need for eco-friendly and cost-effective strategies for sediment treatment, the relevance of this study is justified.

13.2 Methodology

13.2.1 Sample Collection

The collection of sediment samples was obtained at the Asa River, located in Ilorin, North Central Nigeria (8° 28'N, 4° 38′E to 8° 31'N, 4° 40′E), which receives effluent from major industries in the city of Ilorin. The sediment samples were collected from four different points on the river, Coca-Cola (an area that was not dredged), Unity (dredged), Post Office (dredged), and Amilegbe (dredged), and were properly labelled as C, U, P, and A, respectively. Samples were collected into clean polythene bags using a hand trowel and mixed to ensure uniformity, which was then dried in the air for 48 h and sieved through a 2 mm mesh. The organic amendments (rice husk and abattoir effluent) were collected from the National Cereals Research Institute Badeggi, Bida, and Ilorin Abattoir Centre, Ipata Market, Ilorin, respectively, and were properly kept in clean containers to avoid contaminants.

13.2.2 Physicochemical Properties of Sediment

The sediment was analysed for particle size, pH, nitrogen, organic carbon, organic matter, acidity, available phosphorus, and cation exchangeable capacity, as described by Adegbite et al. (2018). Metals such as calcium, magnesium, sodium, potassium, lead, nickel, cadmium, chromium, cobalt, copper, zinc, manganese, and iron were analysed with the aid of an atomic absorption spectrophotometer (AAS) (Perkin Elmer 200 AAS).

13.2.3 Microbial Analysis

13.2.3.1 Fungi Isolation

Fungi were isolated from the Asa River sediment and the two organic amendments (abattoir effluent and rice husk) using a serial dilution method. The sediment sample (10 g) was introduced into 90 mm of sterile water in a conical flask and shaken vigorously. Tenfold serial dilutions were thereafter carried out in sterile water. The 10^{-3} dilution (1 mL) was plated out on sterilized potato dextrose agar (PDA) using the pour plate method. After 5 days, the fungal colonies growing on the plate were counted. Pure cultures of isolates were made on freshly prepared PDA and incubated at 28 ± 2 °C for another 5 days. Stock cultures were made on PDA slants in McCartney bottles and stored in the refrigerators for further microbial analysis. The same process was repeated for each of the organic wastes (abattoir effluent and rice husk).

13.2.3.2 Identification of the Isolates

Microscopic identification was confirmed at the Department of Crop Protection, International Institute of Tropical Agriculture (IITA).

13.2.3.3 Morphology Characterization

Colony and microscopic morphologies were employed for fungal isolates based on the colony appearance on the PDA. The cultural characteristics of fungal isolates, such as mycelia growth form, spore presence or absence, pigmentation, and back colour of colonies on PDA, were also recorded.

13.2.3.4 Microscopic Features

Each fungal isolate was mounted on a slide with a cotton blue lactophenol stain. Microscopic features were observed at × 40.

13.2.4 Experimental Bioremediation

As a method to collect sediment samples, the top 0.25 m of the river bed was dredged from the bottom of the Asa River. Laboratory-scale bioremediation tests were performed in a microcosm.

13.2.4.1 Bioremediation Consortium for Microbes

Soil samples were taken from a number of possible contamination sites along the Asa River's industrial zone. The soil samples were mixed and cultured in a culture medium containing 2 g of NH_4Cl, 0.25 g of NaCl, 0.2 g of sulphur, 0.2 g of $MgSO_4.7H_2O$, and 0.5 g of KH_2PO_4 in 1 L of H_2O. Every 5–7 days, 250 mL of fresh medium was added to 2 mL of a fully established culture in an Erlenmeyer flask. The pollutants added during the acclimatization stage provided the carbon source. A rotary shaker with a temperature setting of 350 °C and 250 rpm was used to provide the microorganisms with the mineral nutrients they require to survive and grow.

13.2.4.2 Procedure for Acclimation

Roughly one millilitre of the culture was moved to a new solution after a 7-day interval. After that, 0.01 g of the sediment sample was placed in a 24-h culture at 350 °C and 250 rotations per minute. A total of 0.5 g of sediment was added to the tank after 0.1 g of sediment was added at the expiration of the first day, which permitted the cell population to develop adequately. This process was repeated incrementally until 0.5 g of sediment had been added in total. The consortium was used in bioremediation studies after several enrichment steps (109 CFU/mL). It was also put to the test to see if it could use organic molecules in sediments under aerobic conditions.

13.2.4.3 Developing an Experimental Design

In six pans, each with a surface area of 498.1 cm^2 and a capacity of 1785 cm^3, the sediment was agitated weekly with a sterile spatula to supply it with appropriate oxygen and air. To achieve a sediment moisture level of roughly 60% of the microcosms' water retention, aluminium foil was placed over the pans and maintained at room temperature (28 ± 2 °C). Deionized water was then added every week until the sediment moisture content was achieved.

All treatments were subject to these conditions. They were as follows:

- Treatment 1: A three-time autoclave at 121 °C for 30 min was used to sterilize the sediment in Pan 1 (control).
- Treatment 2: In Pan 2, no nutrients or culture supplements were applied. Biostimulation was performed with simple aeration.
- Treatment 3: It was determined if biostimulation could be achieved by aeration and addition of nutrients to Pan 3, which contains urea, $(NH_4)_2SO_4$, and K_2HPO_4 at a ratio of 100:10:1.
- Treatment 4: An experiment was conducted with bioaugmentation using aeration and nutrients for Pan 4, which was treated with nutrients and a 50 mL inoculum of 3.2×10^9 CFU/mL of a previously enriched microbial consortium from toxic soil.
- Treatment 5: Pan 5 received 50 mL of abattoir effluent for bioaugmentation with aeration and abattoir effluent.
- Treatment 6: Pan 6 was evaluated with bioaugmentation using aeration and rice husk, which received 50 g of rice husk.

13.2.5 Biological Activity in Different Treatments Was Measured Using Basal Respiration, Dehydrogenase Activity, Microbial Biomass, and Phytotoxicity

Biological activity was assessed by measuring variables such as microbial biomass carbon, basal respiration, dehydrogenase activity, and dissolved oxygen content in the sediments of the treatment units at 0, 2, 4, 6, 8, and 12 weeks. To monitor the above-mentioned parameters, sample composites were collected from various parts of the microcosm, and the analytical procedures are listed below.

13.2.5.1 Basal Respiration

In order to measure the CO_2 concentration in sediments, 2 g of sediment samples were collected from different treatment units and placed in sealed plastic vials inside 1 L glass jars. The NaOH trap used in each jar contained 10 mL of 0.2 N NaOH to trap CO_2 released as a result of substrate mineralization. The NaOH trap was periodically replaced. A titration with 0.1 N HCl was used to determine the amount of CO_2 generated by each microcosm after adding 10 mL of $BaCl_2$ to the NaOH trap.

13.2.5.2 Dehydrogenase Activity

The frequency of decrement of 2,3,5-triphenyltetrazolium chloride into triphenylformazan, as described by Alef (1995), was used to measure the dehydrogenase activity. After 24 h, dehydrogenase activity was determined as micrograms of formazan per gram of soil and reported as a percentage of control activity (100%).

13.2.5.3 Plant Toxicology

To test the phytotoxicity of sediment on sorghum, the sediment extract was centrifuged (at 6000 rpm) and filtered with a No. 42 Whatman filter paper. The sediment extract was extracted by adding water to reach 85% moisture content. A diluted extract was made in distilled water and placed into six petri dishes with 10,000 seeds of sorghum in each. Each dilution was incubated at 27 °C for 72 h in the dark.

Equations (13.1–13.3) were used to calculate the GI based on the number of germinated seeds in the sample and the root elongation compared to the control:

$$\text{The relative seed germination percentage} = \frac{\text{Number of seeds germinated in the extract}}{\text{Number of seeds germinated in the control}} \times 100 \quad (13.1)$$

$$\text{Elongation of roots relative to root length in control } (\%) = \frac{\text{Root length in extract}}{\text{Root length in control}} \times 100 \quad (13.2)$$

$$\text{Germination index } (\%) = \frac{\text{GsLs}}{\text{GcLc}} \times 100 \tag{13.3}$$

Here, Gs and Gc are the average numbers of seeds that germinated in the sample and in the control replication, respectively, while Ls and Lc are the average amounts of root length in the sample and in the control replication.

13.2.5.4 Microbial Biomass (Fumigation and Extraction)

Microbial biomass carbon: ethanol-free chloroform was used. For each sample, two subsamples were made: one non-fumigated sample (10 g) for immediate extraction with 0.5 M K_2SO_4 and, in addition to the fumigated specimen (10 g), a non-fumigated subsample was placed for 3 days in a desiccator. 50 mL of 0.5 M K_2SO_4 was added to each subsample and then shaken for 30 min. After shaking, it was filtered through 0.5 M K_2SO_4 on pre-leached Whatman No. 1 filter paper, and the extract was stored in the freezer.

In a vacuum desiccator, 50 mg of the fumigated sample was placed into 50 mL glass beakers, each marked with a pencil. Sharpe operates in chloroform, so the beakers were placed inside a vacuum desiccator. Beakers were stacked in the desiccator by layering them with a vented plate. After placing boiling chips in a 50 mL scintillation vial and adding 30 mL of chloroform in the desiccator, the vial was evacuated and kept in darkness for 3 days (darkness prevents chloroform from breaking down). In each sample, 50 mL of K_2SO_4 was added, and it was shaken for 30 min. Then each sample was filtered through 1.25 mm Whatman No. 1 filter paper that had been pre-leached with 0.5 M K_2SO_4. A wet oxidation method was used to determine the total organic carbon in the extract, as described by Sánchez-Monedero et al. (1996).

13.2.5.5 Determination of the Rate at Which the Isolate Detoxifies Toxic Elements

To ensure that the sediment was free of organisms, 100 g of sediment was measured into nine different glassware containers and autoclaved at 121 °C for 30 min at a pressure of 15 lbs. Three mycelial discs of each of the fungal isolates were inoculated into the sterilized sediment along with nutrients. It was moistened at 60% and properly covered with a plug. For 12 weeks, the experiment was kept at 282 °C.

13.2.6 Analysis of Data

The ANOVA method was used to analyse the data obtained from the bioremediation experiments. The treatment means were separated according to the LSD method at a 5% probability level.

13.3 Results and Discussion

13.3.1 Physical and Chemical Analysis of the Sediment

Table 13.1 shows some analysis of the physical and chemical characteristics of the Asa River sediment used for the study. The sediment had a high level of organic carbon, organic matter, nitrogen, and cation exchangeable capacity. The result obtained in the study indicates that the sediment is acidic. Ogunwale and Azeez (2000) attributed the pH values of sediment to the nature of the parent materials on which the sediment is developed. The high organic matter content can be attributed to sewage, industrial wastes, and agricultural chemicals such as fertilizers, pesticides, and minerals, which are the primary causes of surface water pollution. It is widely recognized that rivers can become contaminated by traces of metal from numerous and diverse sources, which makes them rich in soil nutrients required for plant uptake (Adekola and Eletta 2007). The ECEC (64.20 Cmol/kg), the overall value of sodium, calcium, magnesium, potassium, and exchangeable acidity (0.65 Cmol/kg), present in the Asa River sediment was found to be high, which showed a high fertility status and its potential for agricultural use. However, anthropogenic activities, such as quarry sites and industrial effluents along the riverbank, still remain the principal cause of the increased amount of heavy metals that have been dumped into the water (Adekola et al. 2002), which have made the Asa River sediment a pool of heavy metals.

Table 13.1 Physicochemical properties of sediment collected from Asa River sediment

Parameter	Values
Particle size (%)	
Sand	75.96
Silt	11.28
Clay	12.76
Textural class	Sand
pH in H_2O	6.02
pH in KCl	5.14
Total organic carbon (%)	0.834
Total organic matter (%)	1.441
Total nitrogen (%)	0.37
Available phosphorus (P) (mg/kg)	23.7
Potassium (K) (Cmol/kg)	24.94
Sodium (Na) (%) (Cmol/kg)	23.83
Calcium (Ca) (Cmol/kg)	6.32
Magnesium (Mg) (Cmol/kg)	8.46
Exchangeable acidity (Cmol/kg)	0.65
Effective cation exchangeable capacity (ECEC) (Cmol/kg)	64.20

13.3.2 Microbial Analysis of Asa River Sediment and Organic Amendments

The pH of Asa River sediment, its organic content, and water are the main factors affecting the fungal population and diversity in the sediment, which is similar to the report made by Yu et al. (2007) that physical-chemical properties had a great impact on the fungal population. Fungi require organic carbon, nitrogen, phosphorus, and potassium. Mould development and sporulation, in addition to those of other microbes, are severely impeded in the absence of any of these (Saksena et al. 1995). According to reports, the monsoon (rainy) season, when soil moisture was noticeably high, was when the fungal population was at its highest level. According to Deka and Mishra (1984), the dispersion of mycoflora is significantly influenced by environmental parameters such as pH, moisture, temperature, organic carbon, organic nitrogen, and organic carbon. In the present study, 21 fungal species were observed (Fig. 13.1c). *Ascomycotina* and *Zygomycotina* had the highest number of fungal species observed. The report of the study shows that *Aspergillus niger* (15.4%), *Aspergillus flavus* (11.1%), *Aspergillus sydowii* (6.1%), *Aspergillus terreus* (4.7%), *Aspergillus glaucus (4.1%), Trichoderma harzianum (7.6%), Penicillium notatum, and Trichoderma viride* (4.5%) were the dominant species, and *Botryodiplodia theobromae* has the lowest occurrence (1.5%); they show vigorous growth and were found in large numbers. It was discovered that a variety of parameters, including temperature, humidity, vegetation, organic and inorganic materials, soil type, and texture, influence the frequency of mycoflora in various fields. Figure 13.1a depicts the proportion of fungus found in abattoir wastewater. There were 11 fungal species identified. *Microsporum nanum* had the highest rate of incidence (16.6%), whereas *Aspergillus niger* had the lowest rate (3.7%). Figure 13.1b depicts the frequency of occurrence of fungi identified from rice husk. A total of 14 fungus species were identified. *Aspergillus flavus* was the most common (14.6%), while *Stachybotrys chartarum* was the least common (2.2%).

Table 13.2 shows some important features of isolated fungal species based on their macroscopic and microscopic appearances. Figures 13.2, 13.3, 13.4, and 13.5a depict some of the characteristics of the potato dextrose agar (PDA) plate. Figures 13.2, 13.3, 13.4, and 13.5b show the conventional method's microscopic appearance at × 100. The morphology characterizations were done based on the colony's appearance, colony form, elevation, colony margin, and colour.

13.3.3 Bioremediation Experiment

13.3.3.1 Respiratory Activity in Microcosms

Figure 13.6a shows the rate at which the organisms present in each microcosm released CO_2 during the respiration process. Treatment A (the control pan) had the least value, probably because it was autoclaved at a very high temperature, leaving no room for organisms to survive at the commencement of the study, but at week 12, the value increased to 1.533, which may be attributed to exposure to

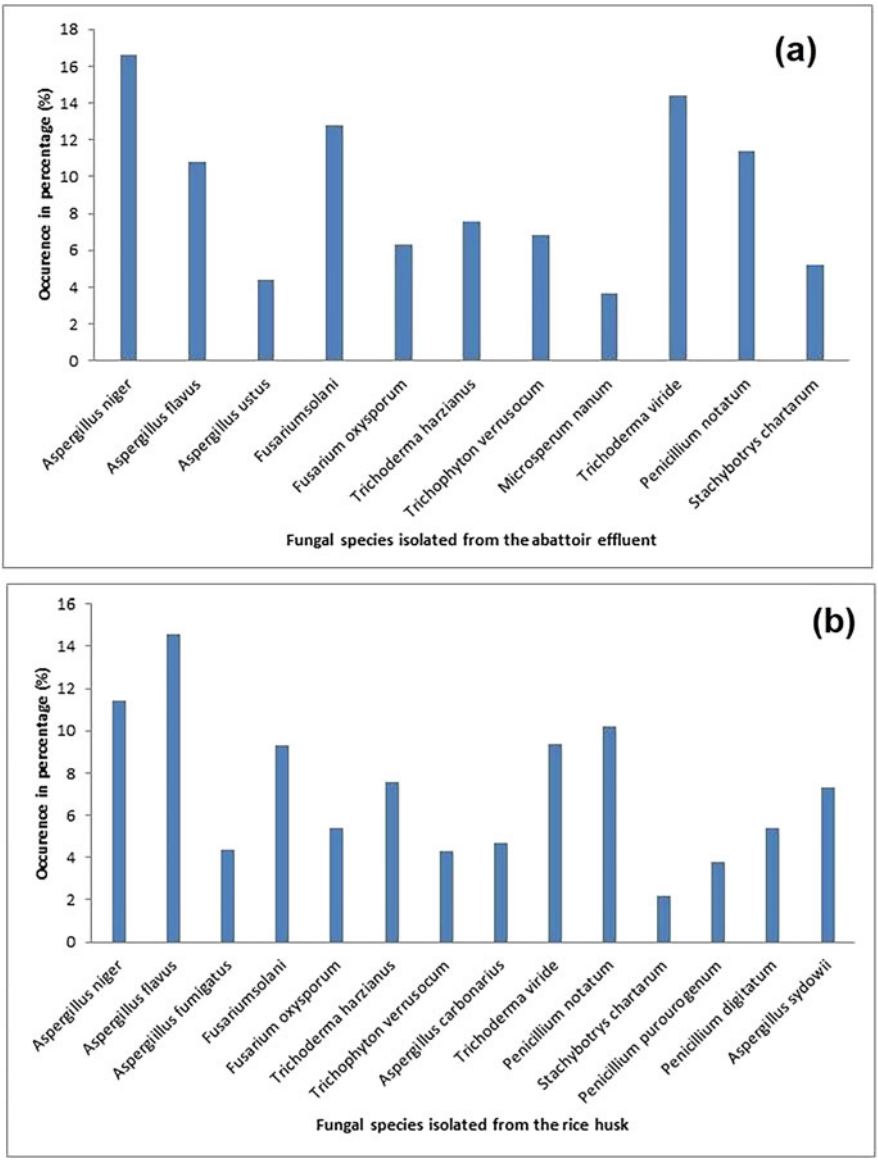

Fig. 13.1 Occurrence of fungi in (**a**) abattoir effluent, (**b**) rice husk, (**c**) Asa River sediment

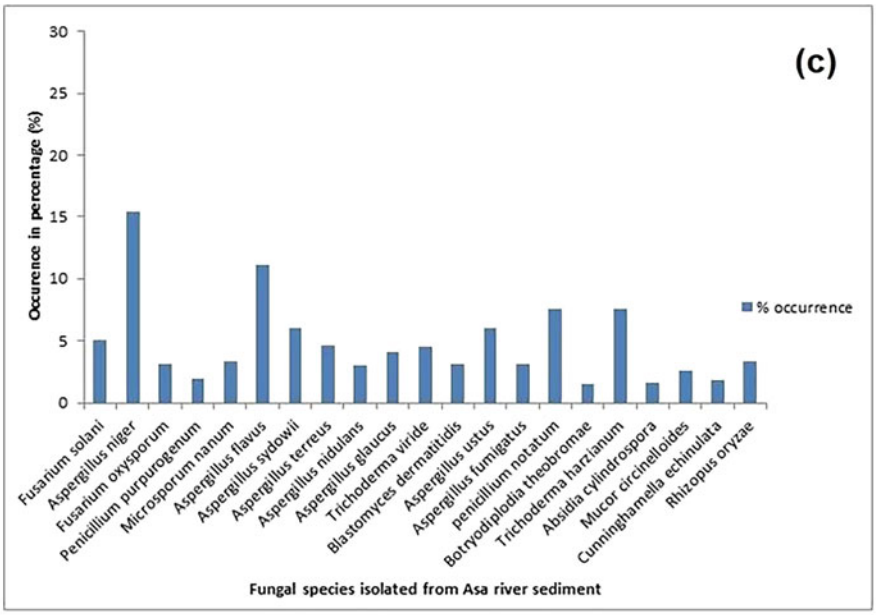

Fig. 13.1 (continued)

contamination from the air during the collection of sediment samples for analyses. The results of microbial activities in the different microcosms are shown in Fig. 13.6a. Treatments are shown comparing respiration rates in non-amended and modified sediment (amended) from the same location. Some trends can be observed: in almost all the treatments investigated, the respiration rate was higher in the modified sediment (biostimulation and bioaugmentation). This is probably due to increased organic nutrients from the microcosm, nutrient addition, or organic waste (rice husk and abattoir effluent) present in the pans. The reduction in CO_2 released thereafter could be a result of a reduction in microbial activity. The microbial activity might have dropped due to nutritional limitations. Engelberg-Kulka and Hazan (2003) demonstrated that bacteria and fungi cells that have begun the sporulation process postpone endospore production by eliminating their peers and feasting on the resources released as a result. They discovered that under nutritional stress, fungi such as *Aspergillus niger* exhibit cannibalistic behaviours. It was also observed that organisms in treatment 5 (nutrients with abattoir effluent) released more CO_2 than other treatments until the 10th week before dropping below treatments 6 and 4. The exception to this trend is the control, in which the respiration rate was 1.533 lower in the contaminated sediment. This may be due to the lack of aeration or addition of nutrients to reactivate the microbes present in the sediment. The action of the sediment microflora is indicated by basal respiration, which may be connected to the decomposition of the molecules in the sediment of the Asa River, which corresponds with the study by Bhattacharyya et al. (2001), where metabolic data

Table 13.2 Colony and microscopic morphology of fungal isolates

ID	Source	Colony morphology	Microscopic morphology	
A_1	Sediment	On PDA, the growth is rapid white aerial mycelia, which become tinged with purple colour and might be masked by cream to tan to orange sporodochia	Microconidia abundant, generally single celled, oval to kidney shaped, macroconidia abundant, only slightly sickled shaped, thin walled, and have attenuated apical cell	*Fusarium oxysporum*
A_2	Sediment	Colonies on PDA at 27 °C attained a diameter of 4–5 cm within 7 days, consisting of a compact white or yellow basal felt with a dense layer of dark brown to black conidiophores	Conidia heads, black, radiate, tending to split into columns with age. Conidiophore stipes smooth-walled hyaline but also in brown colour. Phialides borne on metulae, 7.0–9.5 × 3.5 μm, metulae brown often septate, 15–25 × 4.5–6.0 μm. Conidia globose to subglobose	*Aspergillus niger*
B_3	Abattoir effluents	Colonies with loose white to yellow mycelium rapidly becoming dark brown to black on the development of conidia	Conidial heads are large (3 mm by 15–20 μm in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidial heads are biseriate with the phialides borne on brown, often septate	*Aspergillus niger*
A_3	Sediment	Growth rate: rapid, the texture is silky to coarsely fluffy, radial grooves, the colour is white and reverse is deep yellow. Variants are slow growing, heaped and folded, yellow surface, no reverse pigment, macroconidia absent; reverts to typical colony on rice grains	Long, rough, thick-walled macroconidia with asymmetrical knob on end few pyriform microconidia, lateral racquet hyphae, nodular bodies	*Microsporum canis*
B_4	Abattoir effluents	Growth rate is slow and its texture is waxy or glabrous, heaped or flat, thallus colour is white, grey or yellow and the reverse is colourless, variants, flat, downy, grey-white	Irregular hyphae with many terminal and intercalary chlamydospores. Chlamydospores are often in chains. The head of some hyphae is broad and club shaped, and occasionally divided. The occasional strains produce clavate to pyriform microconidia	*Trichophyton verrucosum*

(continued)

Table 13.2 (continued)

ID	Source	Colony morphology	Microscopic morphology	
			borne singly along the hyphae	
B_8	Rice husk	On potato dextrose agar, colonies are slow growing, small, button or disk shaped, white to cream coloured, with a suede-like to velvety surface, a raised centre, and flat periphery with some submerged growth. Reverse pigment may vary from non-pigmented to yellow	Irregular hyphae with many terminal and intercalary chlamydospores. Chlamydospores are often in chains. The head of some hyphae is broad and club shaped, and occasionally divided. The occasional strains produce clavate to pyriform microconidia borne singly along the hyphae	*Trichophyton verrucosum*
A_6	Sediment	Very rapid rate of growth, maturing in about 3 days. Surface is greenish-yellow to olive and may have a white border, usually consisting of dense felt yellow-green conidiophores. Conidial heads typically radiate, later splitting into several loose columns. Texture is often floccose, especially near the centre, and overall can be velvety to woolly. Unremarkable cream to tan to yellowish reverse	Conidiophores hyaline coarsely roughed, up to 1.0 mn (some isolates are up to 2.5 mm) in length. Phialides borne directly on the vesicle or metulae, 6–10 × 4.0–5.5 μm, metulae 6.5–10 × 3.5 μm. Conidia globose to subglobose	*Aspergillus flavus*
C_2	Rice husk	Colonies have fast-growing, suede-like to downy, white with yellowish green conidial heads. Colonies become greyish-pink to brown with age	Conidiophores are hyaline, smooth walled and bear terminal verticils of 3–5 metulae, each bearing 3–7 phialides. Conidia are globose to subglose, 2–3 μm in diameter, smooth walled and are produced in basipetal succession from the phialides	*Penicillium marneffei*
A_7	Sediment	Growth rate is moderate. Colour is influenced by media. It has a blue-green to dark green to greyish-turquoise. Colonies may have straw-coloured to reddish-brown shades with exudate. Reverse is maroon. Texture is lanose (woolly)	The long, smooth-walled stipes which bear the conidiophores are hyaline generally. The vesicles (7.0–17 μm wide) may appear sub-spherical or clavate. Conidiogenous structures are biseriate with metulae (2–3.5 μm × 4–6 μm) and	*Aspergillus sydowii*

(continued)

Table 13.2 (continued)

ID	Source	Colony morphology	Microscopic morphology	
			phialides (2–3 μm × 5–7 μm) in size. Conidia are spinose and are about 2.5–4.0 μm in diameter	
B_1	Abattoir effluents	Colonies growing rapidly, 4.5 cm in 4 days, aerial mycelium white, becoming purple with discrete orange sporidia present strains, reverse hyaline to dark purple	Conidiophores are short, single, lateral monophialides in the aerial mycelium, later arranged in densely branched. Macroconidia are fusiform and slightly curved. The phialides are short and mostly non-septate	*Fusarium oxysporum*
A_8	Sediment	Colonies on PDA appeared as rapidly growing powdery colonies with a characteristic buff or cinnamon-brown colour on the surface and a yellow to beige-brown colour on the reverse	Hyphae are septate and hyaline with conidial head formed of compact columnar and contain metulae, which supports the phialides (biseriate). The conidiophores are smooth walled, with length ranging from 50 to 320 μm long, and terminating in mostly globose vesicles; the conidia are globose, smooth and small with a size of 2–2.5 μm in diameter and the conidia are hyaline, globose, and sessile and are produced on submerge	*Aspergillus terreus*
A_{10}	Sediment	Growth is slow to moderate. Colony size expands rather slowly. Colony colouration is media dependent but is described as a dull to deep green to a greyish turquoise, with yellow to orange areas. The reverse is pale yellow to yellow	The conidia head radiates to loosely columnar; the conidiophores are smooth walled, 15–30 μm; the upper portion of the vesicle is covered by septate, phialides, and hyaline; the vesicles are globose to sub globose, uniseriate with a 3.5–6.5 μm	*Aspergillus glaucus*
B_6	Rice husk	Very rapid rate of growth, maturing in about 3 days. Surface is greenish-yellow to olive and may have a white border; it consists of dense felt yellow-green conidiophores. Conidial heads typically radiate, later splitting into several	Conidiophores hyaline coarsely roughed, up to 1.0 mn (some isolates is up 2.5 mm) in length. Phialides borne directly on the vesicle or metulae, 6–10 × 4.0–5.5 μm, metulae 6.5–10 × 3.5 μm.	*Aspergillus flavus*

(continued)

Table 13.2 (continued)

ID	Source	Colony morphology	Microscopic morphology	
		loose columns. Texture is often floccose, especially near the centre and overall can be velvety to woolly. Unremarkable cream to tan to yellowish reverse	Conidia globose to subglobose	
A_{11}	Sediment	It is a rapidly growing mould which matures in 3–5 days. Growth begins as fluffy white tufts, which then compact and appear woollier. Green tufts may develop within the colony due to the production of conidia. These often appear as concentric rings, typically starting at the edge of the colony. The reverse is typically a light tan to yellow or pale orange	Conidiophores hyaline, much branched, not verticillate; phialides single or in groups; conidia (phialospores) hyaline, one celled, ovoid, borne in small terminal clusters; usually easily recognized by its rapid growth and green patches or cushions of conidia	*Trichoderma harzianum*
C_7	*Rice husk*	The surface growth is velvety, downy, or powdery, showing various shades of green, most commonly a blue-green to a grey-green with a narrow white border. The colour typically darkens with age. The reverse is white to tan to pale yellowish. Colouration or shade can be dependent on the media on which the fungus is cultured upon	Hyphae are septate with smooth-walled conidiophores (usually less than 300 μm in length and 5–10 μm wide)	*Aspergillus fumigates*
B_5	Abattoir effluents	Colonies growing rapidly, 5–7 cm in 5 days, aerial mycelium white to cream, becoming bluish-brown when sporodochia are present	It possesses 3–5 septate, fusiform, cylindrical, moderately curved, a short blunt apical cell, 28–42 × 4–6 μm. Microconidia are usually abundant, cylindrical to oval, 1–2 celled and formed from long lateral phialides 8–15 × 3–4.8 μm, chlamydospores are hyaline, globose. Smooth to rough walled	*Fusarium solani*
A_{13}	Sediment	The surface growth is velvety, downy, or powdery, showing various	Hyphae are septate with smooth walled conidiophores (usually less	*Aspergillus fumigates*

(continued)

Table 13.2 (continued)

ID	Source	Colony morphology	Microscopic morphology	
		shades of green, most commonly a blue-green to a grey-green with a narrow white border. The colour typically darkens with age. The reverse is white to tan to pale yellowish. Colouration or shade can be dependent on the media on which the fungus is cultured upon	than 300 μm in length and 5–10 μm wide). Vesicles are subclavate in shape, roughly 20–30 μm in width. Phialides are flask shaped, uniseriate, compact, usually forming on the upper two-thirds of the vesicle and mature parallel to the axis of the conidiophore. Conidia are verrucose, (sub)spherical, and about 2–3.5 μm in diameter and develop in chains	
		Colonies are flat, spreading, and white to cream coloured, with a dense cottony surface which show some radial grooves. Colonies usually have a bright golden yellow to brownish yellow reverse pigment	Macroconidia are small typically spindle shaped with 5–10 cells, verrucose, thick walled and often have a terminal knob	*Microsporum canis*
A_{14}	Sediment	Colonies may show variation in surface colour from a yellowish brown to a drab olive with possible greys. They may show a lighter coloured outer edge, and droplets of purple exudate may appear on the surface of the maturing colony. The reverse is a yellowish brown colour. Texture was even and rather	It produces septate hyphae from which smooth-walled conidiophore stipes extend. Stipes are short (130–300 μm). Vesicles, subspherical in shape, also were rather small (7–15 μm diameter) from the vesicles, biseriate conidiogenous cells extend with the metulae being slightly shorter than the phialides. The conidiogenous cells produce round conidia (3.0–4.5 μm diameter) bearing a noticeably rough wall	*Aspergillus ustus*
B_6	Abattoir effluents	Colony grows fast on PDA, white-pinkish on surface and cream reverse	Conidiophores arising from a single or less often synnemata branched near the apex, penicillate, ending in phialides: conidia hyaline, one celled, mostly globose or ovoid, in dry basipetal chains	*Penicillium viride*

(continued)

Table 13.2 (continued)

ID	Source	Colony morphology	Microscopic morphology	
A_{17}	Sediment	The colony is flat, downy to cottony, covered by greyish, short, aerial hyphae. The reverse side is typically brown to black due to pigment production	It has a septate, dark hypha. Conidiophore is also septate and sometimes has a zigzag appearance. It bears simple or branched large conidia (8–16 × 23–50 μm), which have both transverse and longitudinal septations. These conidia are observed in acropetal chains and may produce germ tubes. They are ovoid to obclavate, darkly pigmented, muriform, and smooth. The end of the conidium nearest the conidiophore is round while it tapers towards the apex. This gives the typical beak or club-like appearance of the conidia	*Alternaria alternate*
A_{18}		Light yellow, moist appearance, red with cottony and orange-brown mycelium, with light brown exudates; it grows between 3 and 5 days on PDA	It has a long, thin with about 100 μm or more phialide; it has a single or pairs of chlamydospore; the microconidia is 8–16 × 2–4 μm with abundant, cylindrical, dorsal, and ventral surface parallel, 3–5 septa (35–55 × 4.5–6 μm)	*Fusarium solani*

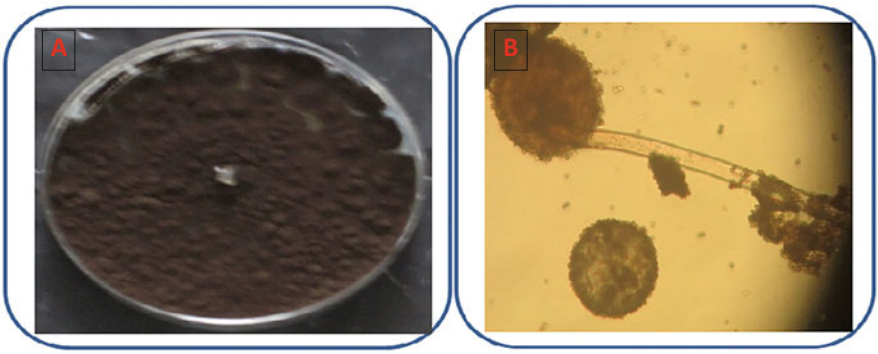

Fig. 13.2 (**a**) Colony morphology and (**b**) microscopic structure of *Aspergillus niger* ×100

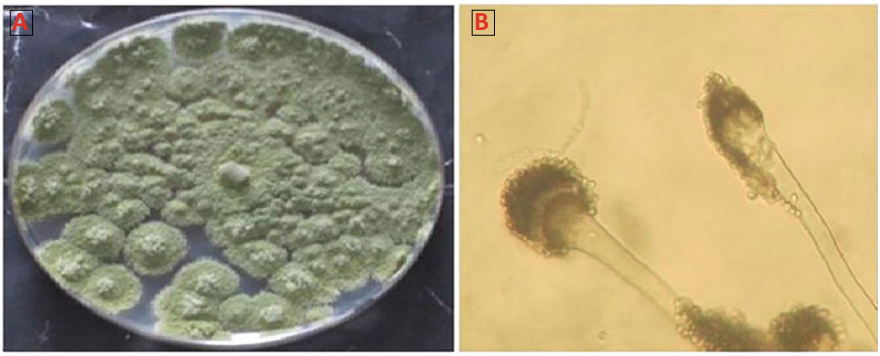

Fig. 13.3 (**a**) Colony morphology and (**b**) microscopic structure of *Aspergillus flavus* ×100

Fig. 13.4 (**a**) Colony morphology and (**b**) microscopic structure of *Fusarium oxysporum* ×100

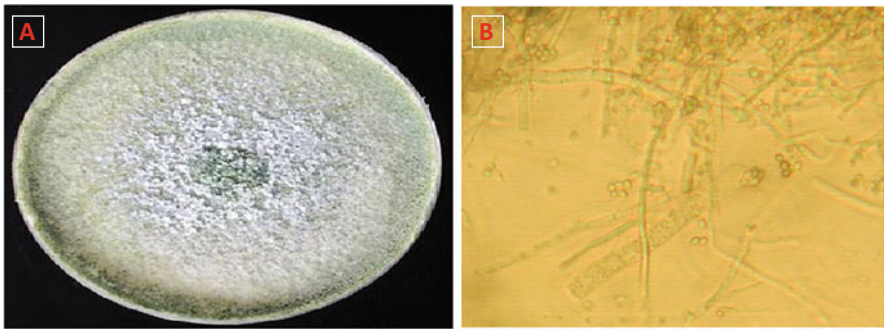

Fig. 13.5 (**a**) Colony morphology and (**b**) microscopic structure of *Trichoderma harzianum* ×100

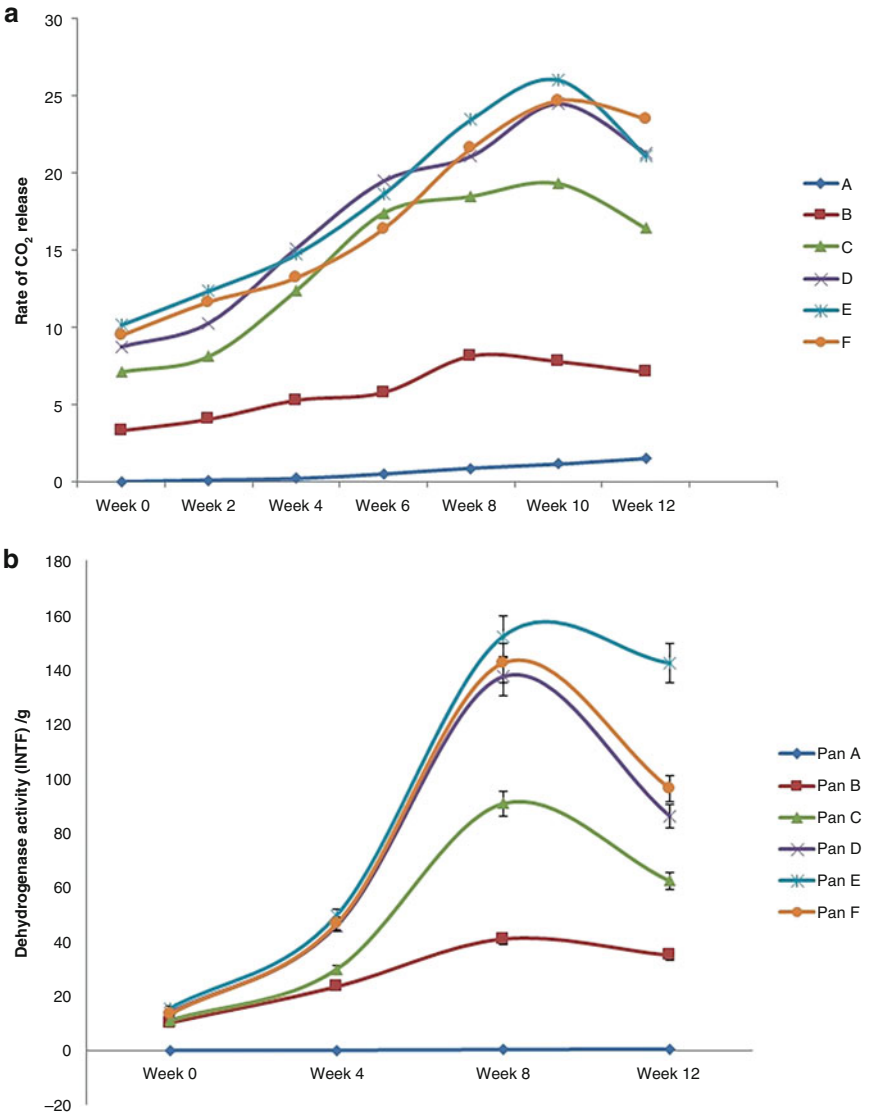

Fig. 13.6 Changes in (**a**) basal respiration with different treatments. (**b**) Dehydrogenase activity in different treatments. (**c**) Germination index (GI) of seeds with time within different treatments. (**d**) Microbial biomass with time within different treatments

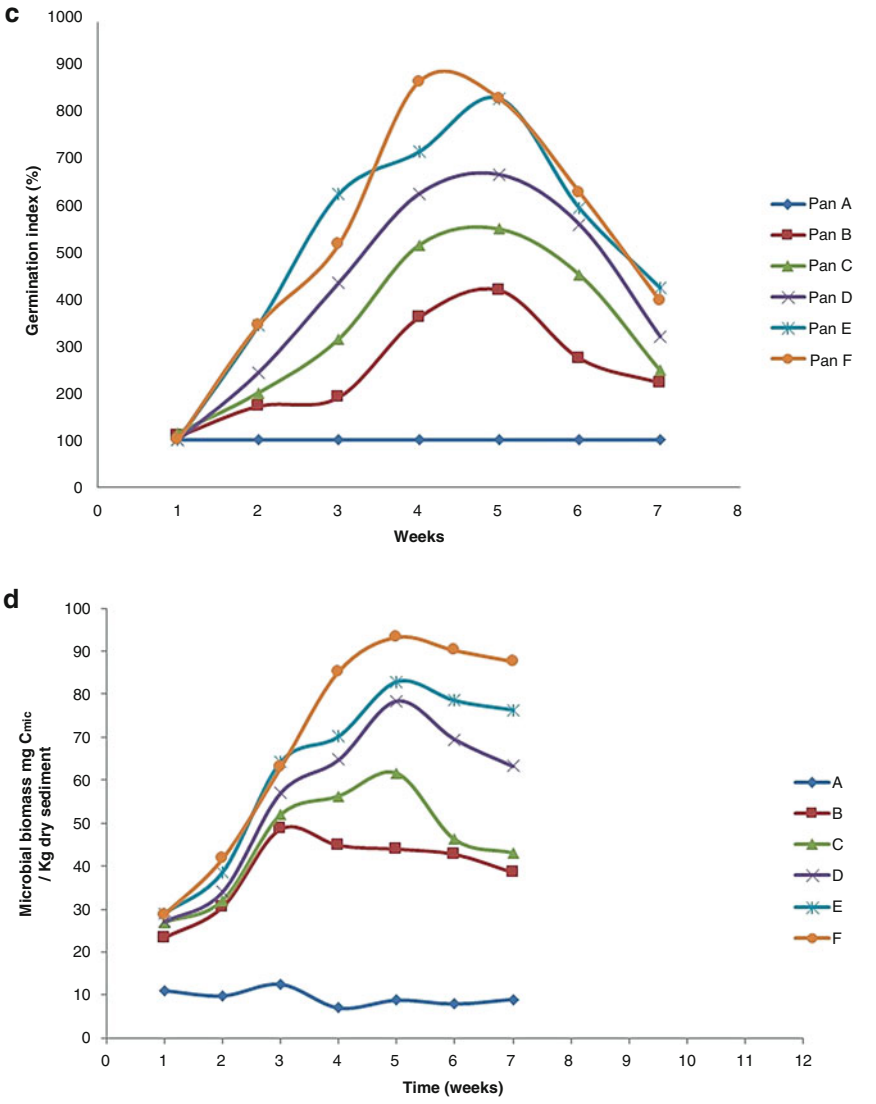

Fig. 13.6 (continued)

are used to estimate the amount and kind of substances that are amenable to mineralization as well as the biological growth in the sediment.

13.3.3.2 Dehydrogenase Activity (DHA)

The relative activity of dehydrogenase throughout treatment is seen in Fig. 13.6b. Treatment 5 (nutrient and abattoir effluent) had the largest microbial activity, as shown by the elevated dehydrogenase activity as a result of sediment moisture

content; DHA is increased when the moisture is high, and water availability in the abattoir effluent strongly affects soil microbial activity and composition (Chander and Brookes 1991). This could be a major reason for the increase in microbial numbers (Bhattacharyya et al. 2001). Thus, the organic amendment increases the organic matter content, which directly increases the enzyme activity. By week 8, the incorporation of rice husk and an improved microbial community had raised the dehydrogenase activity from 15.4 to 152.4 μg INTF/gm dw. In treatment 6, which had nutrients and rice husk, the dehydrogenase activity increased from 13.5 to 142.57 μg INTF/gm dw by week 8. Surprisingly, the quality of the OM in the soil is more significant than its quantity since OM impacts the energy source for microbial development and the manufacture of enzymes (Aoyama and Nagumo 1997). The addition of an enriched microbial consortium in treatment 4 also had a great effect. Biostimulation with nutrient addition increased dehydrogenase activity, but at a lesser rate when compared to pan with a microbial consortium. The activity of the enzyme was enhanced by biostimulation with aeration alone (treatment 2), with a small rise in dehydrogenase activity.

DHA levels in all treatment units were observed to decrease after 8 weeks, most likely as a result of the degradation of labile organics. Bioaugmentation (pans D, E, and F) had a higher rate of dehydrogenase activity than the treatments with biostimulation (B and C) and control. The nutrients (energy) in pans D, E, and F revive the microbes present in them for optimum dehydrogenase activity. In this regard, Aoyama and Nagumo (1997), Nweke et al. (2006), and Nweke et al. (2007) established that little modification of the sediment increases the energetic metabolism, which increases the enzymes' activities.

13.3.3.3 Phytotoxicity Assay

Table 13.3 shows the results of sorghum seeds that germinated after 24, 48, and 72 h. It was observed that the germination average on all amended treatments is higher than that of the non-amended treatment (control). Figure 13.6c demonstrates that after the addition of an organic amendment and an enriched colony of microorganisms, the phytotoxicity degree in the sediment has been significantly lowered (treatments 5 and 6). This was soon followed by treatment 4, and a comparably lesser drop in phytotoxicity was found in treatment 3, which boosted indigenous microbial activity through the incorporation of oxygen and supplements. Treatment 2 got biostimulation with aeration only, which resulted in a slight rise in its germination index. Controls exhibited poor GI values that did not alter over treatment, but they also had low germination percent and root length when compared to treatments 5 and 6, where the microorganisms received nutrition for their energy metabolism.

The observations were obvious after week 12, when the amendments had decomposed. Trace element levels were shown to have a considerable ($p < 0.05$) phytotoxic impact on the germination index (Fig. 13.6c). The phytotoxicity of germination and root length improved considerably with a decrease in toxic element concentrations. The results of this finding indicated that heavy metals at levels above the required amount had significant phytotoxicity on sorghum seed germination

Table 13.3 Effects of different treatments on germination rates of seeds

	A (week 0)	A (week 12)	B (week 0)	B (week 12)	C (week 0)	C (week 12)	D (week 0)	D (week 12)	E (week 0)	E (week 2)	F (week 0)	F (week 12)
24 h	0 of 10	1 of 10	1 of 10	2 of 10	1 of 10	3 of 10	0 of 10	3 of 10	0 of 10	2 of 10	0 of 10	3 of 10
48 h	2 of 10	3 of 10	2 of 10	6 of 10	3 of 10	5 of 10	0 of 10	6 of 10	0 of 10	7 of 10	1 of 10	6 of 10
72 h	2 of 10	5 of 10	2 of 10	7 of 10	3 of 10	7 of 10	2 of 10	8 of 10	1 of 10	10 of 10	2 of 10	9 of 10
Average after 72 h (%)	20	50	20	70	30	70	20	80	10	100	20	90

characteristics. Toxic materials' detrimental effect on shoot and root length is directly related to their toxicity on the sorghum germination index. This study's results are in line with those of Radha et al. (2010) and Shaikh et al. (2013), who found that the phytotoxicity of toxic metals on GI was reduced at lower doses and rose at higher doses.

All of the pollutants contained in the treatments significantly ($p < 0.05$) impacted the GI of sorghum. Root lengths and germination rates were both negatively impacted by elevated heavy metal concentrations, and a significant drop was mostly seen at week 12 when concentrations were compared to the control treatment (Table 13.3). Excess salt deposition in the cell wall may reduce germination rates and root lengths by adversely altering metabolic functions and limiting cell wall flexibility (Naseer 2001). Along with causing chromosomal abnormalities and aberrant mitosis, heavy metals may also interfere with cell division, which would impede root growth (Bhattacharyya et al. 2001). According to other studies on the bioassay tests (Kapanen and Itävaara 2001), the results showed the same differences between the control, where the GI was lower, and the amended sediment (bioaugmentation and biostimulation), where the GI was highest.

13.3.3.4 Microbial Biomass

The microbial biomass carbon was determined after week 12. The result as seen in Fig. 13.6d showed that treatment A (sterile sediment) fluctuated at weeks 6, 8, 10, and 12 most likely due to fluctuations in microbes generated by microbial succession. Organic wastes have the effect of the treatment increasing the C_{mic} values, which are significantly higher than those of biostimulation. Beyond week 8, when the microbial biomass was seen to diminish more slowly than in treatment 3, the effects of the microbial consortium and organic wastes were clearly obvious in treatments 4, 5, and 6.

13.3.4 Chemical Analysis of Microcosm

Figure 13.7a–i shows the chemical analysis of different microcosms. A significant decrease in the heavy metal concentration was observed in different treatment units (B, C, D, E, and F). During the 12 weeks of incubation, treatment E (bioaugmentation with abattoir wastewater) demonstrated the ability to eliminate the pollutant the most. However, some of the loss could have resulted from volatilization, as shown in control samples.

In all the figures:
Pan A—control
Pan B—simple aeration
Pan C—biostimulation
Pan D—bioaugmentation (consortium)
Pan E—bioaugmentation (abattoir effluent)
Pan F—bioaugmentation (rice husk)

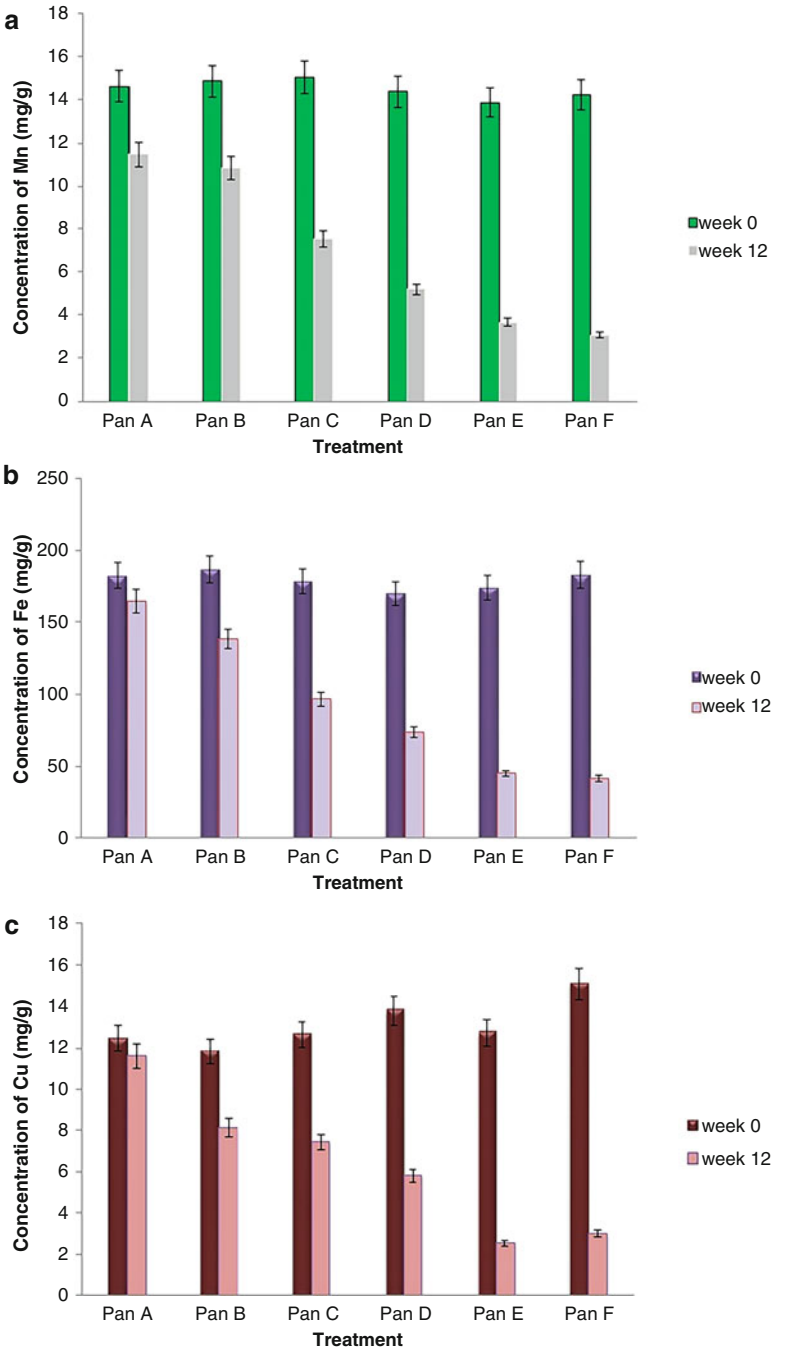

Fig. 13.7 Rate of degradation of (**a**) manganese, (**b**) iron, (**c**) copper, (**d**) zinc, (**e**) cobalt, (**f**) chromium, (**g**) cadmium, (**h**) nickel, and (**i**) lead in different treatments

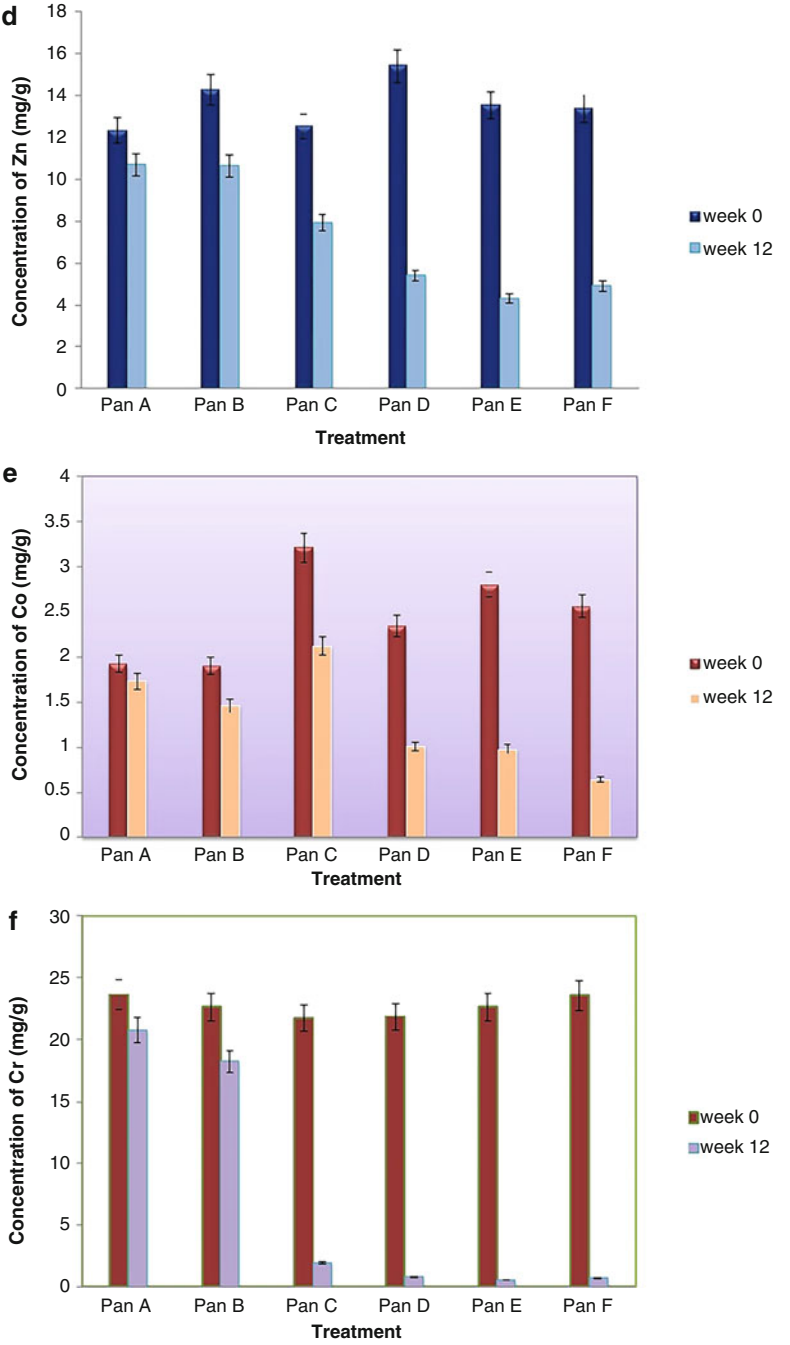

Fig. 13.7 (continued)

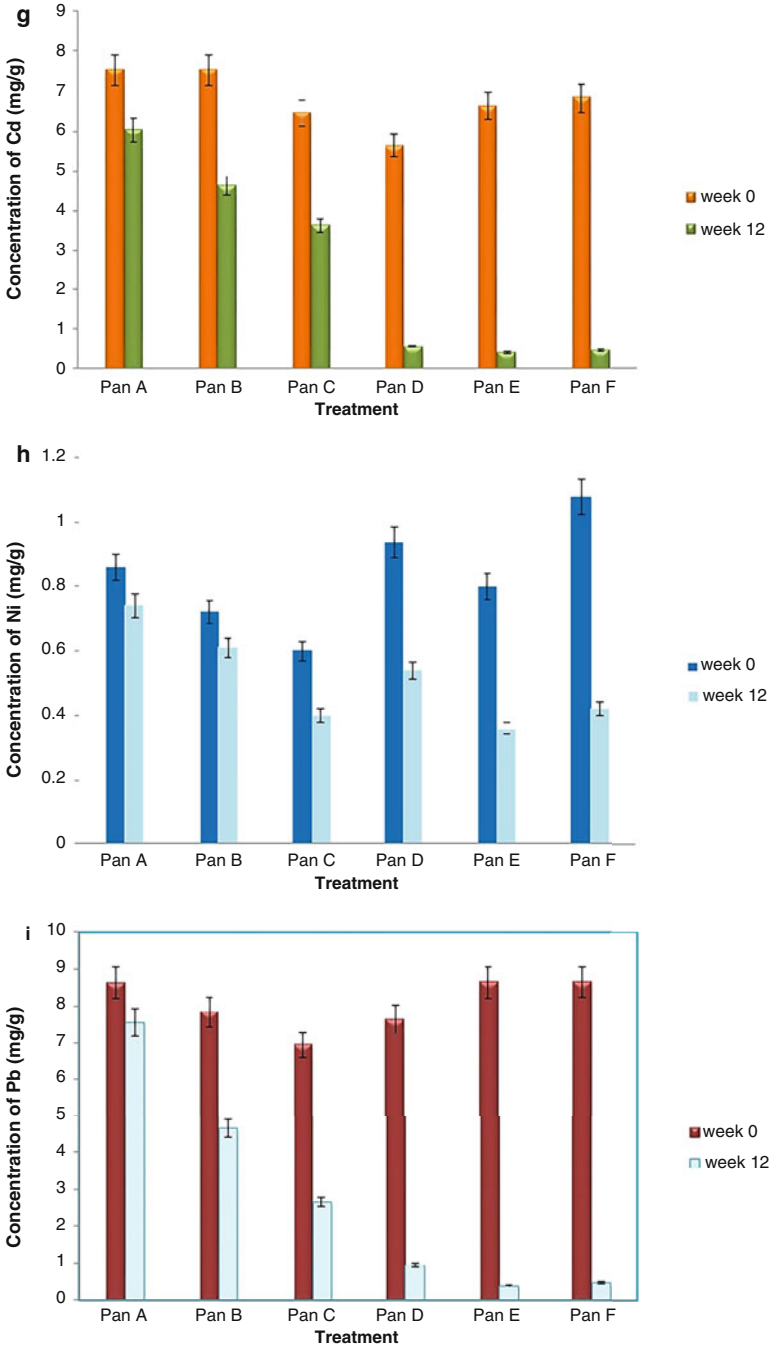

Fig. 13.7 (continued)

13.3.5 Effect of Some Fungal Isolates on the Toxic Elements in Asa River Sediment

Microorganisms isolated from Asa River sediment with substantial metal levels and organic waste show a strong resistance to these components. This resistance may be owing to some abiotic conditions or to the microorganism's biochemical and genetic responses. Romero et al. (1999) observed that numerous microbes have been evaluated for their heavy metal adsorption ability, but those isolated from the Asa River sediment, where these pollutants are prevalent, are of particular relevance in this study. Fungi possess a very-well-known heavy metal sorption capacity. The result showed that some fungal species are commonly connected with substrates that have high concentrations of heavy metals and can even be regarded as heavy metal hyperaccumulators, as previously described by Gadd (1997).

The *Fusarium* sp. showed good tolerance level behind *Aspergillus niger* of sediment samples. There was at least one isolate from each of the two major groups of fungi. Figure 13.8(a–i) illustrates *Aspergillus* sp. with varying degrees of metal tolerance, which was investigated in this study. The isolates from river sediment show that some fungi (*Aspergillus* sp., *Penicillium*, etc.) from heavy metal-contaminated river sediment have higher resistance to heavy metals than others and might be used to mitigate these metals from solutions. Gadd (2007) remarked that due to their intracellular metal sorption nature and resistance to metals, filamentous fungi are more fitted for this function than other microbes. Heavy metals may be amassed by various fungi by a variety of processes, such as polypeptide binding.

Before inoculating samples with various fungal isolates, the sediment contained a high concentration of toxic elements. Figure 13.8a–i shows the differences in the value of heavy metals present before and after 12 weeks of inoculation. *Aspergillus* spp. was found to be among the most prevalent in the heavy metal-polluted sediment, which was similar to the results obtained by Ahmad et al. (2005) and Nweke et al. (2006). Various *Aspergillus* spp. such as *A. niger*, *A. fumigatus*, and *A. flavus* have been engaged in the sorption of heavy metals. Nweke et al. (2007) and Tunali et al. (2006) also observed similar results in their studies. Gadd (2007) investigated the elimination of hazardous elements by *Aspergillus* sp. from untreated wastewater. According to Nweke and Okpokwasili (2013), Cd, Cu, and Ni were the metals to which *Aspergillus* strains were most tolerant. As a result, *A. tamarii, A. flavus, A. niger,* and *A. terreus* were chosen to be studied in the current investigation. *Aspergillus niger* had a higher degradation for most of the heavy elements present in the sediment, except nickel, where *Fusarium solani* had the highest degradation. It was also observed that all the fungi significantly decreased the metal level when compared with the control.

The sediment had a high content of toxic element before inoculating samples with different fungal isolates. Figure 13.8(a–i) shows the differences in the value of heavy metals present before and after 12 weeks of inoculation with varying fungal isolates. *Aspergillus niger* had a higher degradation for most of the heavy elements, except nickel, where *Fusarium solani* had the highest degradation. It was also observed that

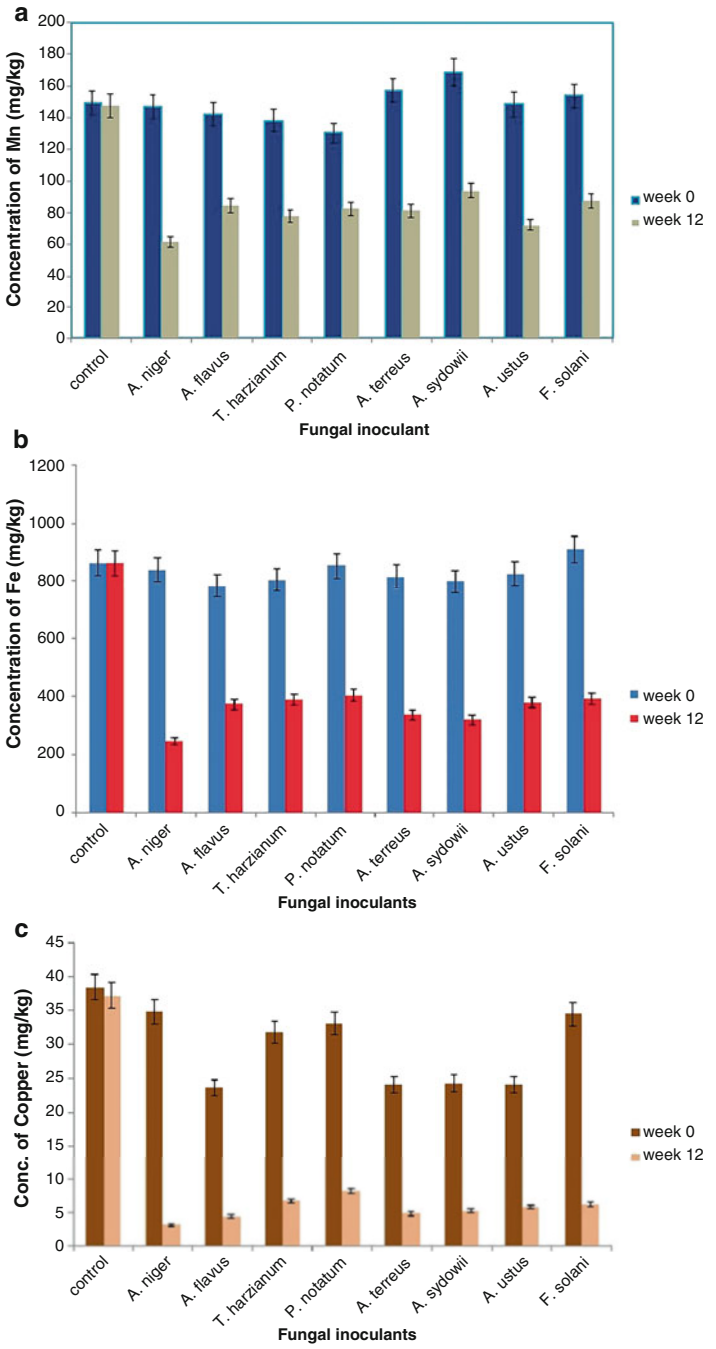

Fig. 13.8 Effect of fungal inoculants on (**a**) manganese, (**b**) iron, (**c**) copper, (**d**) zinc, (**e**) cobalt, (**f**) chromium, (**g**) cadmium, (**h**) lead, and (**i**) nickel levels in Asa River sediment

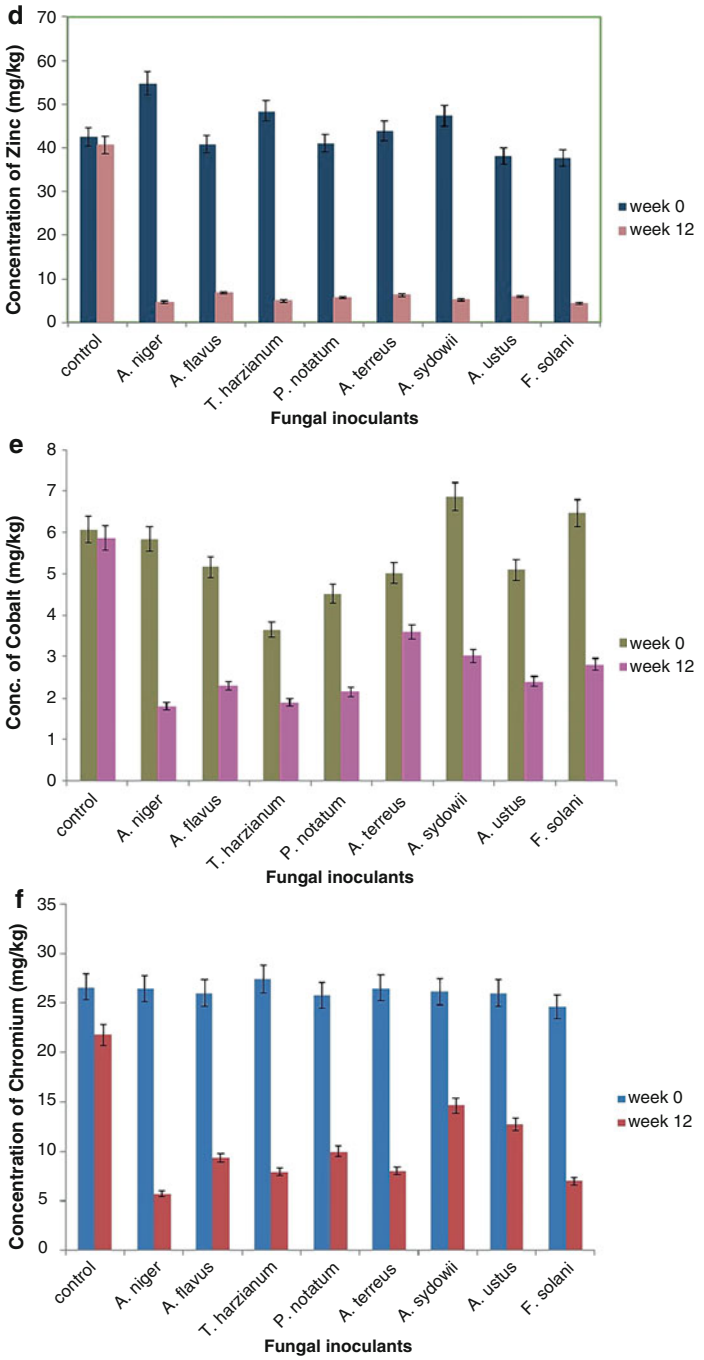

Fig. 13.8 (continued)

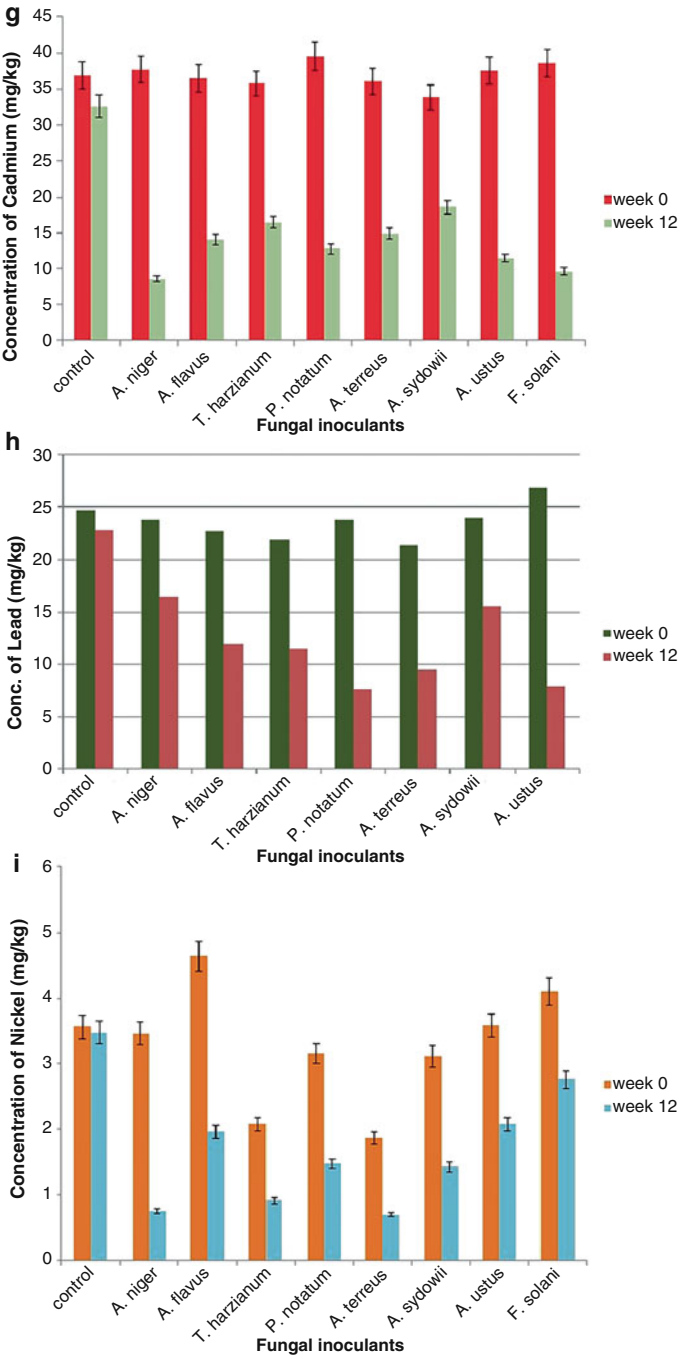

Fig. 13.8 (continued)

all the fungi species led to a significant decrease in heavy metal content when compared with the control.

Figure 13.8a indicates that Mn is degraded in the following order*: A. niger* > *A. ustus* > *A. terreus* > *A. sydowii* > *T. harzianum* > *F. solani* > *A. flavus* > *P. notatum.*

Figure 13.8b indicates that Fe is degraded in the following order*: A. niger* > *A. sydowii* > *A. terreus* > *F. solani* > *A. ustus* > *P. notatum* > *A. flavus* > *T. harzianum.*

Figure 13.8c indicates that Cu is degraded in the following order*: A. niger* > *F. solani* > *A. flavus* > *A. terreus* > *T. harzianum* > *A. sydowii* > *A. ustus* > *P. notatum.*

Figure 13.8d indicates that Zn is degraded in the following order*: A. niger* > *T. harzianum* > *F. solani* > *A. sydowii* > *A. ustus* > *P. notatum* > *A. terreus A. flavus.*

Figure 13.8e indicates that Co is degraded in the following order: *F. solani* > *A. flavus* > *P. notatum* > *T. harzianum* > *A. sydowii* > *A. niger* > *A. ustus* > *A. terreus.*

Figure 13.8f indicates that Cr is degraded in the following order: *A. niger* > *F. solani* > *T. harzianum* > *A. terreus* > *A. flavus* > *P. notatum* > *A. ustus* > *A. sydowii.*

Figure 13.8g indicates that Cd is degraded in the following order: *A. niger* > *F. solani* > *A. ustus* > *P. notatum* > *A. flavus* > *A. terreus* > *T. harzianum* > *A. sydowii.*

Figure 13.8h indicates that Pb is degraded in the following order: *A. ustus* > *P. notatum* > *A. terreus* > *T. harzianum* > *A. flavus* > *F. solani* > *A. niger* > *A. sydowii.*

Figure 13.8i indicates that Ni is degraded in the following order Ni*: A. niger* > *A. sydowii* > *A. terreus* > *A. flavus* > *T. harzianum* > *P. notatum* > *A. ustus* > *F. solani.*

13.4 Conclusion

This work evaluated the use of agricultural wastes (rice husk and abattoir effluent) to bio-remediate the sediment of the Asa River. The physicochemical and heavy metal analyses revealed high concentrations of organic carbon, organic matter, and heavy metal concentrations, among others, in the river sediment. It was observed that higher concentrations of the metals were very phytotoxic ($p < 0.05$) and prevented crop germination. This showed that human activities such as sewage disposal, industrial effluents, and chemicals from agricultural practices are the major causes of the river's sediment and low soil fertility. A total of 21 fungi were isolated from the samples and the agricultural wastes, and they all successfully degraded the heavy metal concentrations, with *Aspergillus niger* being the most effective. This study presents bioremediation as a low-cost and environmentally benign technique for remediating Asa River sediment.

Statements and Declarations

Conflict of Interest The authors declare that there are no conflicts of interest.

Funding There was no external funding for the study.

Human and Animal Rights This chapter does not contain any studies involving human or animal subjects.

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