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Effect of sand mining on the heavy metal and mycoflora status of Teenama creek

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ABSTRACT The heavy metals and mycoflora status of Teenama creek in Rivers state Nigeria was investigated for rainy seasons of industrial scale sand mining operation. The concentration of Arsenic, cadmium, Chromium and Lead were investigated for a period of three years, 2011-2013 from June to September each year. The mycoflora in the creek was also investigated in terms of its fungal count and the genera. The concentration of the investigated heavy metals was 0.130mg/L – 0.491mg/L, 0.144mg/L – 0.345mg/L, 0.432 mg/L – 0.747 mg/L and 0.068 mg/L - 0.221 mg/L for Arsenic, Cadmium, Chromium and Lead respectively. All the investigated metals were above WHO recommended limits and the control. The result indicated a steady increase in heavy metal concentrations as the sand mining persisted. Fungal populations were very low with a constant reduction as the sand mining progresses. The counts decreased steadily from 4.8×10^2 CFU/ml in the year 2011 to 2.0×10^0 CFU/ml in the year 2013. The fungal average count in the control site range was 6.5×10^4 CFU/ml to 6.8×10^4 CFU/ml during the study period. Only 8 species from 7 genera of fungi were isolated from the sand mining sampling stations. The control station had 32 fungal species from 23 genera. *Aspergillus spp. Candida albicans, Cladosporium herbarum, Fusarium spp* and *Penicillium spp.* are implicated as pathogens. The Margaleef and Shannon Weiner Indices showed that the Teenama creek had depleting fungal species. There were Significant Difference between the sampling site and the control at $P = 0.05$ and 0.01 Level of Significance. The Teenam creek is polluted with heavy metals and not suitable for the purposes for which it is used by the populace. The Biological end-point is steadily reducing drastically indicative of danger for other life forms within the creek.

Keywords: Teenama creek, sand mining, fungi, heavy metals, Lead, Arsenic.

Introduction

Sand by way of definition is a loose material, incoherent mass of mineral materials; a product of natural processes, such as, weathering or denudation etc. Because of their origin (rock) the primary component of sand are quartz and silica (SiO_2).

Sand mining is a coastal activity referring to the actual removal or excavation of sand from the ocean and coastal water floor (Ashraf *et al.*, 2010). Sand mining may also be extended to streams in Nigeria. This is achieved using various kind of sand mining/gravel extraction operation like dry-pit mining, wet-pit mining, bar skimming or scalp (use of machine) mining and manual methods. The manual method is mostly subsistence as a source of income to most unemployed youths.

However, the use of machine is an industrial activity with high economic value. This is due to its demand for all kind of construction projects such concrete, filling of roads, building sites, brick making, glass making, sand paper etc. The mining of sand can generate environmental abnormalities depending on method of extraction, intensity, duration and geological feature of the point of extraction. Environmental problem arises when the rate of extraction of sand and other materials exceed the rate of natural processes generating them in such environment (Ashraf *et al.*, 2010).

All this contribute to varying concentration of chemical content especially heavy metals in the water column. It is well known that some of these metals possess potential toxicity to microbes and other life forms within the ecosystem. More so trace metals are important for growth metabolism in living cell at low concentration and microorganism possess mechanism of varying specificity for inter-cellular metal accumulation from external environment; for example, Copper (Cu), Zinc (Zn), Iron (Fe), Nickel(Ni), and Cobalt(Co) (Pradipta, 2008).

Heavy metals are metallic elements that have a relatively high density compared to water. In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals. Reported sources of heavy metals in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources (He, *et al*., 2005). Environmental pollution is very prominent in point source areas such as mining, foundries and smelters, and other metal-based industrial operations (He, *et al*., 2005).

In biological systems, heavy metals have been reported to affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair. Metal ions have been found to interact with cell components such as DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell cycle modulation, carcinogenesis or apoptosis (Beyersmann and Hartwig, 2008). Several studies have demonstrated that reactive oxygen species production and oxidative stress play a key role in the toxicity and carcinogenicity of metals such as arsenic (Yedjou and Tchounwou, 2007), cadmium, chromium (Patlolla, 2009; Patlolla et al. 2009) and lead (Yedjou and Tchounwou, 2008). Because of their high degree of toxicity, these elements rank among the priority metals that are of great public health significance. They are all systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. According to the United States Environmental Protection Agency (U.S. EPA), and the International Agency for Research on Cancer (IARC), these metals are also classified as either “known” or “probable” human carcinogens based on epidemiological and experimental studies showing an association between exposure and cancer incidence in humans and animals.

The fishermen of Teenama and neighboring settlements reported death of fishes, prawn and crabs along the shores and in the mangrove swamp of the creek during low tide. Preliminary investigations revealed there was ongoing industrial scale sand mining in the creek which was the only industrial activity within the environment. The necessity arose to study the creek water for its heavy metal status and an accompanying biological indicator of pollution.

Exposure of creek water to re-suspended sediments and heavy metals due to sand mining may result in toxicity to primary producers and consumers, leading to death of creek animals and plants, as were observed at site during the study.

Mining of sand and other mining processes is a major source of anthropogenic input of heavy metal in aquatic environment beside industrial process and sewage sludge. The active process of sand mining increases the mobilization of heavy metals.

Microbial populations are nutrient dependent and are affected by the concentration of anions, cations and heavy metals (Obire *et al*., 2008; Obire and Barade, 2009; Obire *et al*., 2008). Surface growth of entomopathogenic fungi was strongly inhibited by heavy metal ions. Their response to heavy metal ions depended not only on the examined ion but also on the species and strain of fungus. Surface growth of the tested *Paecilomyces farinosus* strain was strongly inhibited by the heavy metal ions. The ions of lead, cadmium, zinc, and copper were particularly toxic. The results of Ropek1 and Para (2003) implied that the Cu, Pb and Cd ions were the most toxic for the investigated *P. farinosus* strain. The tested heavy metal ions affected growth, sporulation and pathogenicity of *P. farinosus* differently, but Pb and Cd ions proved toxic for growth, sporulation, and pathogenicity of *P. farinosus* (Ropekl and Para, 2003).

It is justifiable to use Fungi as the biological end-point following the documended evidence of the response of their growth and sporulation to heavy metal concentration. Hence it is necessary to study changes in fungal frequencies during the period of sand mining with the associated heavy metals in the creek.

Materials and Methods

The Study Area

The study site where water samples were obtained is the Teenama Creek at the outskirt of Ken-khana, close to Nyorgorong and located close to the Khana -Andoni boundary at magnetic coordinate 04°34’24N and 007°27’18E in Rivers State Nigeria.

Teenama Creek is a marine ecosystem with tidal flux, and also have link to other inland water bodies in Khana. It has mangrove vegetation ranging from plankton to angiosperm as well as a diversity of animal community. The creek configuration widens in some region and narrows in some other areas.

Sample Collection

Five stations were chosen in two different creeks to evaluate heavy metal pollution of site or sampling points. Four (4) sampling points were chosen within the Teenama creek dredging site and a control sampling point in Baghan Boue creek. The water samples were collected in plastic bottles from June to August, for four years (2011-2014) at the respective sites. The fifth site, Baghan Bo-ue creek served as a control.

Sample W_1 was collected from the main channel by the bridge, W_2 was collected from a point branching from the main channel into the mangrove swamp, W_3 was collected from artificial pond water produced at the dredged sand sedimentation site and sample W_4 collected at the location of the dredger in the main channel of Teenama creek. W_5 was collected from the main channel of Baghan Bo-ue creek as control.

The samples were collected in plastic containers rinsed properly with distilled water and at site with water from collection point. All samples were taken by wading into the water at low tide to collect sub-surface water. The appropriately labeled samples were kept in cool condition (between $10^{O}C$ – 15 ^{O}C) and moved to the laboratory for analysis.

Analytical grade reagents were used for standard preparation. Analysis were done in triplicates, back-up with blank determination. The heavy metal concentration of the digested samples was determined by comparing their absorbance with those of the standard – solution of known metal concentration using APHA 3111B (Shimadzu AAS 6650). Arsenic (As) was determined using hybrid generation Atomic absorption spectrometry (APHA 3114B).

Fungal Cultivation, Enumeration, Characterization and Identification

Czapek Dox Agar was prepared in accordance with the modification of Czapek solution by Smith (1971) in agar. A 0.1ml aliquot of 10^{-1} dilution was plated onto agar plates and incubated at $27.5^{O}C$ in an inverted position for 5 days. The discrete colonies that developed were counted and the mean of replicate plates were recorded. The counts were also computed into colony forming units of fungi per ml (CFU/ml).

Fungal cultures were observed while still on plates and after wet mount in lactophenol on slides under the compound microscope. The observed characteristics were recorded and compared with the fugal identifications keys of Malloch (1997).

Statistical Analysis

Identified fungal genera and species were subjected to Shannon-Weiner diversity index and Margalef species richness index to compare the Teenama creek water sampling points and the control.

Result

The result of the range and mean values of the concentration of heavy metals (Arsenic, Cadmium, Chromium and Lead) of the different sampling sites of Teenam creek water and the control site is shown in Table 1 below. The measured concentrations were compared with that of the control site and WHO maximum permissible limits for water.

Table 1: Range and mean values of the concentration of heavy metals (mg/L) in Teenama creek water

Sampling Site	Concentration of heavy metals (mg/L)			
	Arsenic	Cadmi-	Chromi-	Lead
W_1	**(0.135 – 0.177) 0.1595**	**(0.168 – 0.197) 0.1803**	**(0.443 – 0.722) 0.581**	**(0.068 – 0.098) 0.082**
W_2	**(0.130 – 0.159) 0.1475**	**(0.153 – 0.182) 0.167**	**(0.432 – 0.588) 0.512**	**(0.071 – 0.093) 0.83**
W_3	**(0.412 – 0.491) 0.4543**	**(0.288 – 0.348) 0.314**	**(0.689 – 0.47) 0.713**	**(0.188 – 0.221) 0.211**
W_4	**(0.138 – 0.175) 0.1598**	**(0.144 – 0.194) 0.1698**	**(0.644 – 0.715) 0.678**	**(0.190 – 0.218) 0.199**
W_5 (control)	**<0.001**	**<0.001**	**<0.003**	**<0.001**
WHO STD	**0.01**	**0.003**	**0.05**	**0.01**

The minimum value of Arsenic concentration was 0.130mg/L and the maximum value was 0.491mg/L. Sampling site W_2 had the least average concentration (0.1475mg/L); while the highest average concentration was 0.4543mg/L in sample W_3. The measured arsenic concentrations in the sand mining sites were higher than the control site and WHO maximum permissible limits (0.01mg/L) in water.

The minimum concentration of Lead (Pb) was 0.153mg/L in 2011 and the maximum value was 0.348mg/L. Sampling site W_2 had the least average concentration (0.1670mg/L); while the highest average concentration was 0.314mg/L in sample W_3. The measured cadmium concentrations in the sand mining sites were higher than the control site and WHO maximum permissible limits (0.03mg/L) in water.

The minimum concentration of Cadmium (Cd) was 0.153mg/L in 2011 and the maximum value was 0.348mg/L. Sampling site W_2 had the least average concentration (0.1670mg/L); while the highest average concentration was 0.314mg/L in sample W_3. The measured Cadmium concentrations in the sand mining sites were higher than the control site and WHO maximum permissible limits (0.03mg/L) in water.

The minimum value of chromium concentration was 0.432mg/L in 2011 and the maximum value was 0.747mg/L in the year 2014. Sampling site W_2 had the least average concentration (0.512mg/L); while the highest average concentration was 0.713mg/L was in sampling site W_3. The measured chromium concentrations in the sand mining sites were higher than the control site (0.03mg/L) and WHO maximum permissible limits (0.05mg/L) in water.

The minimum value of lead concentration was 0.068mg/L in 2011 and the maximum value was 0.221mg/L in the year 2014. Sampling site W_1 had the least average concentration (0.082mg/L); while the highest average concentration was 0.2113mg/L in sampling site W_3. The measured lead concentrations in the sand mining sites were higher than the control site (0.00525mg/L) and WHO maximum permissible limits (0.05mg/L) in water.

The result of the mean values of the fungal count (CFU/ml) of the different sampling sites of Teenam creek water and the control site for the various months is shown in Table 2 below.

Table 2: Mean values of the fungal count (CFU/ml) of the various sites of Teenam creek water and control for the various months of the three years

Month	Fungal count (CFU/ml) in the sampling site				
	W_1	W_2	W_3	W_4	Control
June	8.83×10^1	1.54×10^2	1.0×10^2	1.5×10^2	6.2×10^4
July	7.7×10^1	1.16×10^2	6.2×10^1	1.3×10^2	6.5×10^4
Aug	1.2×10^1	1.4×10^1	1.2×10^1	1.1×10^1	7.3×10^4
Sept	1.3×10^1	1.5×10^1	9.3×10^0	1.2×10^1	7.1×10^4

The result of the mean values of the fungal count (CFU/ml) of the different sampling sites of Teenam creek water and the control site for the various years is shown in Table 3 (on the next page).

Table 3: Mean values of the fungal count (CFU/ml) of the various sites of Teenam creek water and control for the various years

Year	Fungal count (CFU/ml) in the sampling site				
	W_1	W_2	W_3	W_4	Control
2011	1.22×10^2	1.76×10^2	2.31×10^2	1.07×10^2	6.8×10^4
2012	1.3×10^1	1.4×10^1	1.1×10^1	1.5×10^1	6.5×10^4
2013	8.0×10^0	9.0×10^0	6.75×10^0	7.5×10^0	6.6×10^4

Fungal populations were very low with a constant reduction as the sand mining progresses. The mean values of the counts decreased steadily from 2.31×10^2 (CFU/ml) in the year 2011 to 6.75×10^0 (CFU/ml) in the year 2013. The fungal average count in the control site ranged from 6.5×10^4 (CFU/ml) to 6.8×10^4 (CFU/ml) during the study period.

The result of the fungi isolated from Teenama creek and the control site is shown in Table 4 (next page). The few genera that were isolated from the Teenama creek were those tolerant to the toxic effects of some heavy metals.

Table 4: Fungi isolated from the Teenama creek sand mining sites and the control

Fungi species isolated		
Teenama creek	**Control**	
Aspergillus niger	*Aspergillus flavus*	*Mucor globosus*
Aspergillus spp.	*Aspergillus fumigatus*	*Mucor sp.*
Candida albicans	*Aspergillus spp.*	*Penicillium spp.*
Cladosporium herbarum	*Aureobasidium pullulans*	*Rhizopus nigricans*
Fusarium spp.	*Aspergillus versicolor*	*Pestalotia spp.*
Penicillium spp.	*Aspergillus niger*	*Penicillium simplicissimum*
Rhizopus nigricans,	*Blastomyces sp*	*Rhizopus sp.*
Trichoderma lignorum	*Aspergillus wentii*	*Phoma spp .*
	Candida albicans	*Rhodotorula spp.*
	Cephalosporium curtipes	*Saccharomyces sp.*
	Cladosporium berbarum	*Sporobolomyces sp.*
	Cladosporium cladosporioides	*Sporothrix schenckii* *Syncephalastrum racemosum*
	Coccidioides immitis	
	Cryptococcus neoformans	*Trichoderma lignorum*
	Cunninghamella sp.	*Trichophyton sp*
	Fusarium spp.	
	Histoplasma capsulatum	

Discussion

The amount of heavy metal present in the creek water column is significant. The presence of Chromium, Cadmium and Arsenic in the polluted site raises fear of imminent toxicity to human and animals consuming fish from the Teenama creek.

The concentration of Cadmium in the Study area was more than the control and higher than WHO Standards for water. Chronic exposure to Cadmium causes damage to the immune system, psychological disorder, damage to the central nervous system, cancer development, Bone fracture and reproductive disorder. It also has pronounced effects on the kidney. Chromium (IV) for instance is considered a Class I Carcinogen. Chromium (VI) compounds induce oxidative stress, DNA damage, apoptotic cell death, and altered gene expression. It also causes skin rashes, weakened immune systems, kidney and liver damage, alteration of genetic materials, lung cancer and ultimately death. The presence of chromium at the recorded concentration is very significant.

All the heavy metals sampled have exceeded minimum toxicity limits and of very grave concern for fish and other aquatic lives as well as humans, reptile-birds and other animals depending on these fishes for food.

The reason for such damnable condition of Teenama creek water is sediment re-suspension in water through sand mining process since there are no industries discharging effluents into the creek. In addition, the non-degradable nature of heavy metals and possibly slow rate of dispersion into the high sea may lead to the observed significant increase in metal over a short period of time (about 3 years) sand mining started at Teenama. The implication of the finding is that the prevailing effluent limitation standard enacted by regulation agencies since 1999 (Don-Pedro *et al*., 2004) is inadequate and does not cover or affect every industry especially sand mining, hence, the need to review it and implement it as well. This is based on the result obtained so far as control samples occur at acceptable limit while those in the vicinity of sand mining were very high.

The biological significances of observed increase in heavy metal concentration in Teenama creek compared to that of the standard limit by WHO and the control is a danger that can disrupt ecological balance and may lead to the total collapse of the fragile ecosystem, if heavy metal concentration increase with time despite its source. This effect or deleterious effect not only will cause a reduction in microbe, but also a reduction of population of in fauna which if sustained will lead to the total loss of the depleting biological diversity. Moreso, the possibility of bio-accumulation of some of these metals and the flow in the food chain involving plants and animal-species particularly in edible periwinkle (*Tympanotanus fuscatus*), oysters, mudskipper (*Penoptithalmas* spp), and crab (*Portunus pelaguas*) on which the people depend as food and for commerce, could result in the transfer of such toxic metallic load to human consumer (Pradipta, 2008). This may lead to public health problems and may account for most untreatable cases in clinic/hospitals. This establishment calls for the need to include regular biological monitoring using edible accumulator of priority pollutant as indicator species in an effective effort to prevent or minimize environmental and food poisoning is human.

Only 8 species from 7 genera of fungi were isolated and identified from the sand mining sampling stations. The control station had 32 fungal species from 23 genera. These fungal diversity distributions are similar to those obtained in Obire et al 2008 from creek water polluted from industrial activities in Rivers State Nigeria. *Aspergillus spp. Candida albicans, Cladosporium herbarum, Fusarium spp.* and *Penicillium spp.* are causes of mycoses. The situation where these genera thrive in the absence of competition from other genera that can regulate their population is of major concern. It is an indication that the Teenama creek water is not suitable for recreation and any domestic use as a result of the ongoing industrial sand mining operations.

There was highly significant difference between the Teenama creek and the control at P = 0.05 and P = 0.01.

Furthermore, the result provides greater credibility to the call to abolish industrial or large-scale exploitation of sand in creeks as obtainable in other nations of the world like France, Netherland, England etc. as stated by Ashraf *et al*., (2010).

Conclusion

The result obtained established the pollution of the creek water with As, Cd, Cr, and Pb, in the duration of sand exploitation. Samples obtained from W_3 (sand sedimentation pond), indicate the highest concentration of As, Cd, Cr, and Pb, than samples of W_1, W_2, W_4. But that of W_5 (control) were below WHO approved limits. All sampling points within the creek contain heavy metal concentrations above the acceptable limits. The fungal population was undergoing depletion from heavy metal toxicity, showing that the food chain is negatively impacted. Genera of fungi known to cause serious debilitating human diseases were those commonly isolated from the sand mining station, indicative of the high-levels of resilience required for pathogenicity. The creek water is linked to fresh water streams used for drinking and domestic purposes within the region. The neglect of effect of sand mining on coastal waters is a grave negligence as such environment constantly empty into international waters during ebb and tide. Nigeria is expected to regulate and protect its environment as required by international conventions to which it is signatory.

Recommendations

- The regulatory organizations should ensure that Environmental protection laws are strictly adhered to by companies operating in the country.
- The Post Impact assessment of the activities of such industry should be done before commencement of operations
- Regular Environmental Evaluation (Reports EER) should be done to ascertain the environmental status of the area and the effluent receiving environment.
- There is urgent need to commence remediation work on Teenama creek to forestall further degradation of the impacted sites and the attendant health risk to human, animals and plants.
- Efforts should be made to restore the impacted sites to its original pre-impact status.
- Fishing and exploration of the Teenama creek and its efflux waters for any form of marine food be stopped immediately until full remediation of the creek is carried out and its recovery ascertained.

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