

粒径分级叶绿素 a 对富营养水体生物修复的响应

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**摘要:**通过对富营养小水体叶绿素 a 分粒径分析,探讨了生态修复对水体粒径分级叶绿素 a 的影响以及各粒径叶绿素 a 对修复的响应,Nano-粒径叶绿素 a 为本水体的第 1 贡献者,Net-粒径也占有较高的比例,处于第 2 位,Pico-粒径所占份额最小;环境因子 BOD<sub>5</sub> 和 TN/TP 与 Net- Chla %和 Nano-Chla %分别呈非常显著负和正相关;生物修复实施前后 Net-chla %和 Nano-chla %均有非常显著变化,生态修复工程后网采浮游植物相对生物量明显增多,微型浮游植物相对生物量显著减少,而微微型浮游植物相对生物量仅有小幅度的升高,修复前后并无显著差异。

**关键词:**生物修复; 叶绿素 a; 粒径; 富营养

Responses of all size-fractionated chlorophyll-a contents to bioremediation in eutrophic water

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**Abstract:** Using the relationships between organism and it's surroundings have been widely adopted as the the way to evaluate if ecosystem is healthy or not by experts. Many of scholars are attempting to erect a biologic method so that the water quality could be quickly assessed. Provided that a method can be established to quickly assess the presumptive effect for water body. As with the process of bioremediation, it can not only provide the solid proof for scientific management and for selecting correct presumptive plan, but can also facilitate subsequent decision-making process as well.

In the process of bioremediation, by testing all size-fractionated chlorophyll-a in eutrophic water, the article explores how size-fractionated chlorophyll-a is in response to the bioremediation, in a hope to reflect the effect of bioremediation in accordance with the response of all size-fractionated chlorophyll-a, so as to provide biological proof for illustrating the efficiency of measures taken for the bioremediation.

This test water is: water area(350m<sup>2</sup>), water dept(60cm), water volume(210m<sup>3</sup>).

Before testing, spot survey was conducted and found to be in a condition of hypereutrophic. Comprehensive measures concerning ecology are taken including the way of adding Bioenergizer, biologic algaecide, effective microbe into water, and planting Macrophyte. Beforehand, background was sampled twice on 11th and 27th of April. The experiment began from April 29, 2002. And after the engineering project, sampling work was done on 16th, 24th and 30th of May, 06th, 13th, 20th and 27th of June, 04th, 11th, 12th, 13th, 14th, 15th, 17th, 25th and 31th of July respectively.

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Water samples for phytoplankton analyses were collected at the depth of 0~2 m, 0.5m with length Niskin bottles (for a total final volume of 10L). Next, the samples were divided into pico (0.2~2.0 μm), Ultra (0.2~5 μm), Nano (2~20μm), Net (20~200μm) size fractions by filtration. Chlorophyll-a was determined by spectrophotometry following the methods proposed by Lorenzen. Samples for water quality analyses were collected at the integrated depths of 0m with a 0.5 m for determination of temp, DO, pH, SD, COD<sub>mn</sub>, BOD<sub>5</sub>, TN, NH<sub>4</sub>-N, NO<sub>2</sub>, PO<sub>4</sub> and TP.

Within the periods of time in this survey, the average value of the content of the following elements involving Net-, Nano- and Pico-Chla amounts to be 51.486, 83.491, 6.056 and 2.622 mg/m<sup>3</sup> respectively. The average value of contribution rates of Net-, Nano-, Ultra- and Pico-Chla occupied 32.250%, 65.240%, 4.721% and 2.091% respectively. Nano-phytoplankton is the highest contribution to bulk chlorophyll-a contents, Net-phytoplankton also occupies the higher rate, being placed in the second, while Pico-phytoplankton occupies the least.

Bivariate correlate analyze was done between the content of all size-fractionated chlorophyll-a, the percentages of all size fractionated chlorophyll-a contents to bulk chlorophyll-a contents with all environmental factors. The result showed only the percentages of net-chla and nano-chla obviously correlated to the temperature. Considering the influence of temperature, thus, by controlling temperature, partialcorrelate analysis was redone. The result showed that the percentage of Net-Chla correlated negatively with BOD<sub>5</sub> and TN/TP while the percentage of Nano-Chla correlated positively with BOD<sub>5</sub> and TN/TP. As both BOD<sub>5</sub> and COD are indexes reflecting the level of the water contamination, results of the above analysis signified that thepercentages of Nano-and Net-chla were closely correlated on the level of water contamination.

By conducting ANOVE on the percentages of all size fractionated chlorophyll-a before and after the bioremediation, it was found that the percentages of Net-Chla, Nano-Chla were significantly different after the bioremediation than before. The relative biomass of Netphytoplankton increased and the relative biomass of Nanophytoplankton decreased obviously after bioremediation, but the relative biomass of Picophytoplankton only contained small range of going up, there were not marked changes after bioremediation.

The test of size-fractionated chlorophyll-a can reflect primary message of the importance of the nanoplankton or picoplankton. These phytoplanktons, for their short-period reproduction and amazing growth rate, thus, their quantities, biomass and productivity can be taken as important indexes for the water quality. During the research, It was found that the percentage of size-fractionated chlorophyll-a can not only reflect the surrounding changes, but also was able to produce apparent response to these changes. Moreover, since size-fraction can be rapidly and easily tested and reliable conclusion can be made, and the way of the test tends to be identical both home and abroad, thus the research conclusion is highly comparable, Therefore, changes of percentage of all size-fractionated chlorophyll-a can be considered to make judgment on the water quality and remediation process.

**Key words:** bioremediation; Chlorophyll-a; size-fractionation; eutrophication

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越来越多的研究表明,微型浮游生物和微微型浮游生物是生物量和生产力的主要贡献者<sup>[1~3]</sup>,分级生物量(特别是分粒级测定叶绿素)是反映微型生物重要性的主要信息,目前关于浮游植物生物量的分粒级研究,国外做了相当多的工作<sup>[4~10]</sup>,我国学者也曾先后在青岛、胶州湾、厦门西海域、南大洋及台湾海峡水域开展上述研究<sup>[11~15]</sup>。此类研究主要集中于海洋生态系统,对淡水叶绿素a 粒径分级研究较少见<sup>[16]</sup>,关于粒径分级叶绿素对水体生物修复响应的研究则未见报道。

水体生物修复是人类对污染水体的强烈干预,其手段实施的最终结果是恢复水域生态系统的结构与功能特征,再现一个自然的、能自我维持和调节的生态系统。为了从生物的角度讨论生态修复试验结果,本文按照国际通用标准将浮游生物分为网采浮游植物(Netphytoplankton, 20~200μm, 以下记 Net-);微型浮游植物(Nanophytoplankton, 2~20μm, 以下记 Nano-);超微型浮游植物(Ultraphytoplankton, 0.2~5μm, 以下记 Ultra-);微微型浮游植物(Picophytoplankton, 0.2~2μm, 以下记 Pico-)4 个粒级,通过对生态恢复过程中的富营养水体各粒级叶绿素a 测定,探讨了粒径分级叶绿素对水体生物修复的响应,希望能根据各粒级叶绿素a 的响应来反映生态修复效果,以找到阐明生态修复措施有效性程度的生物学依据。

## 1 研究地区与研究方法

### 1.1 水体状况及生态修复工程措施

试验水体面积为350m<sup>2</sup>,水深约60cm,水容量为210m<sup>3</sup>,试验前对试验水体进行的现场调查表明COD<sub>Mn</sub>、BOD<sub>5</sub> 和氨氮含量是

景观娱乐用水C类水质标准(GB12941-91)的3~10倍,水体呈现严重富营养化(表1)。

水体采用生态综合措施,包括往水体中投加生物促生液(Bioenergizer,简称BE)、生物除藻剂、高效微生物、种植高等水生植物等。其中,生物促生液使用剂量为1.5mg/L,次,每两周一次。生物除藻剂使用剂量为0.03oz/m<sup>2</sup>,每两周一次。两者相互间隔一周使用。高效微生物使用PSB光合细菌和硝化细菌,使用剂量为15 mg/L,每两周一次,与生物促生液同时使用。高等水生植物的种植包括凤眼莲(*Eichhornia crassipes*)、睡莲(*Nymphaea tetragona*)、香蒲(*Typha orientalis*)、芦苇(*Phragmites communis*)、鸢尾(*Iris* sp.)、荷花(*Nelumbo nucifera*)等植物。

1.2 研究方法

1.2.1 样品的采集及采样时间 用采水器取水表以下0.5m水层水样10L,现场立即用200μm分样筛过滤,以去除大型浮游动物活动对浮游植物的影响,镜检结果表明,每次水样几乎都没有大于200 μm的浮游植物存在,所以这一处理对叶绿素总含量没有什么影响。过滤后的水样作测定样品,在暗处保存。

试验自2002年4月29日开始,实施前在4月11日和4月27日进行2次本底采样,工程实施后采样时间分别是5月16、24、30日、6月6、13、20、27日、7月4、11、12、13、14、15、17、25、31日。

水质样品取表、中层混合水样,指标有水温、DO、pH、SD、COD<sub>mn</sub>、BOD<sub>5</sub>、总氮、氨氮、亚硝氮、总磷、正磷酸盐。测定方法采用《水和废水监测分析方法》(国家环保局、《水和废水监测分析方法》编委会编1989)中的分析方法进行,由华东师大环境科学系微生物组同学测定。

1.2.2 叶绿素分级及测试方法 200μm和20μm用分样筛过滤,5μm和2μm用混合纤维滤膜过滤,得<200、<20、<5、<2μm的水样,各分级水样分别经Waterman GF/C玻璃纤维滤纸过滤。将带样品的滤纸剪碎后在研钵中加适量90%丙酮研磨至足够细,移入具塞刻度离心管中于暗处静置萃取20~24h后,离心得清液(提取液)定容,分光光度计测波长665nm和750nm处光密度值,然后加入1滴1N的盐酸酸化,再测波长665nm和750nm处光密度值,计算公式为:

$$C = 27.3 \times (Eb-Ea) \times Ve/V$$

式中,C为水样中叶绿素a的含量(mg/m<sup>3</sup>或μg/L);Eb为提取液酸化前波长665nm和750nm处的光密度之差;Ea为提取液酸化后波长665nm和750nm处的光密度之差;Ve为提取液的总体积(ml);V为抽滤的水样体积(L)。

2 结果与分析

2.1 水体叶绿素a的粒径特征

在本次调查时段内Net-Chla、Nano-Chla、Ultra-Chla和Pico-Chla含量的变幅分别为7.68~235.98、47.65~112.95、0.69~24.54和0.08~12.30mg/m<sup>3</sup>;均值分别为51.486、83.491、6.056和2.622 mg/m<sup>3</sup>(表2)。

Net-Chla%、Nano-Chla%、Ultra-Chla%和Pico-Chla%的变幅分别为9.01%~67.40%、32.02%~90.93%、0.60%~18.72%和0.07%~9.39%;均值分别为32.250%、65.240%、4.721%和2.091%(表2)。可见,本水体的叶绿素a第1贡献者为Nano-粒级,Net-粒级也占有较高的比例,处于第2位,Pico-粒级所占份额最小。

2.2 各粒级叶绿素a的动态特征

Net-Chla含量的时间动态表现为上升趋势,峰值在7月25日。Nano-Chla含量属于小幅振荡的平稳格局,总体变化幅度没有Net-Chla含量大,Ultra-和Pico-Chla含量的变化趋势一致,与Net-Chla含量的动态表现一样,也处于上升趋势,高峰在7月13日(图1)。

Net-Chla%的动态表现为上升趋势,高峰在7月25日。Nano-Chla%变化和Net-Chla%正好相反,属于下降趋势,最低谷也在7月25日。Ultra-和Pico-Chla%的变化趋势较一致,7月13日前处于小幅上升趋势,而后又有回落(图2)。

表1 水体修复前各类指标与富营养化评价标准

Table 1 Indexes of the water before bioremediation and the eutrophic level

指标 Indexes	测定值 (mg/m <sup>3</sup> ) Experimental results	对照值 (mg/m <sup>3</sup> ) Contrast	富营养评价标准与 参考文献 Assessment standards and references
总氮 TP	1890	500~15000	极度富营养 Hypereutrophic <sup>[17]</sup>
总磷 TN	520	30~5000	极度富营养 Hypereutrophic <sup>[17]</sup>
COD	12080	12000	重富营养 Hypereutrophic <sup>[18]</sup>
BOD	7500	5000~8000	富营养 Eutrophic <sup>[18]</sup>
叶绿素a Chl-a	122	10~500	富营养 Eutrophic <sup>[17]</sup>

表2 各粒级叶绿素a含量(mg/m<sup>3</sup>)及所占百分比(%)

Table 2 The chlorophyll-a contents(mg/m<sup>3</sup>) of all size-fractionated and percentage(%) of all size-fractionated chlorophyll-a contents to bulk chlorophyll-a contents

指标 Indexes	N	Minimum	Maximum	Mean	Std. Deviation
Net-Chla	18	7.68	235.98	51.49	51.37
Nano-Chla	17	47.65	112.95	83.49	24.85
Ultra-Chla	18	0.69	24.54	6.06	5.92
Pico-Chla	17	0.08	12.30	2.62	3.28
Net-Chla%	18	9.01	67.40	32.25	16.35
Nano-Chla%	16	32.02	90.93	65.24	17.73
Ultra-Chla%	17	0.60	18.72	4.72	4.52
Pico-Chla%	16	0.07	9.39	2.09	2.57

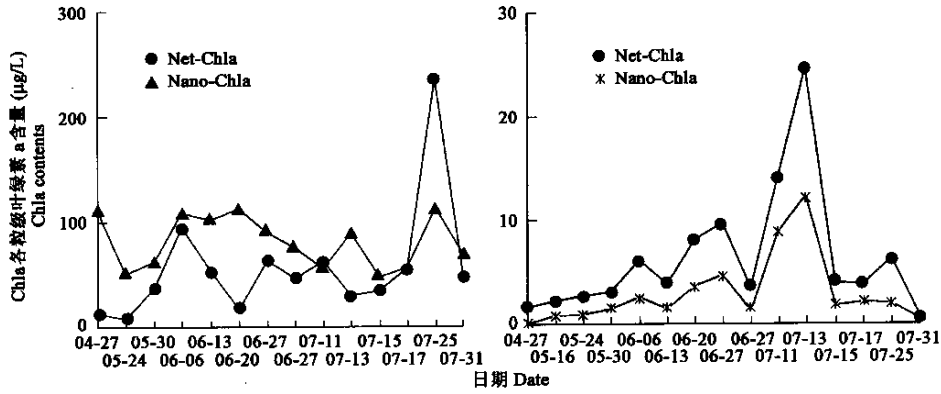


图1 分级叶绿素a的含量(µg/L)的动态

Fig. 1 The variations of chlorophyll-a contents of all size-fractionated phytoplankton

2.3 各粒级叶绿素a与环境因子的关系以及对生态修复的响应

对各粒级叶绿素a含量、各粒级叶绿素a贡献率与环境因子DO、pH、温度、化学需氧量、五日生化需氧量、氨氮、亚硝酸盐、总磷、总氮、总氮/总磷进行相关分析结果表明,除net-chla%和nano-chla%与温度有显著相关外,各生物指标与其它环境因子则无显著相关,考虑到温度对生物和非生物因子的影响<sup>[18]</sup>,以温度为控制因素,再对各生物指标与环境因子作偏相关分析结果为:Net-Chla%与BOD<sub>5</sub>和TN/TP呈非常显著负相关( $P < 0.01$ );Nano-Chla%与BOD<sub>5</sub>和TN/TP呈非常显著正相关( $P < 0.01$ );Net-Chla与COD<sub>mn</sub>和TN/TP呈显著负相关( $P < 0.05$ )。

对生物修复工程实施前与实施后的各粒级叶绿素a贡献率进行方差分析,发现Net-chla含量工程实施前与实施后有显著差异( $P < 0.05$ );Net-chla%和Nano-chla%在工程实施前后有非常显著差异( $P < 0.01$ )。

3 讨论

3.1 分粒级叶绿素a的粒径特征

通过对各粒级叶绿素含量的研究表明,Nano和Pico两个粒级的浮游植物占总生物量的67.3%,微型浮游植物是本研究水体浮游植物现存量的主要贡献者,这一结果与他人研究结果是一致的<sup>[19~21]</sup>。近20a来,关于海洋和湖泊中光合超微藻的分布、组成和食物网动态的研究证明,超微藻类(0.2~2µm)对生态系统浮游植物生物量和初级生产力具有重大贡献。很多超微藻类具有吸收速率极快和独立于细胞域值之外的维持高效吸收能力,这样就使超微藻类在营养物贫乏的水域中和营养物间歇供给的水域具有竞争优势<sup>[22]</sup>。因此,超微藻在水体中的数量和生物量的大小可作为水生生态系统质量的一项重要指标。

Malone就对微型浮游植物与水域营养状况的关系有过报道<sup>[23]</sup>,他认为,从浮游植物的地理分布上看,微型浮游植物是大洋区(贫营养水域)的重要种类,而较大的硅藻、甲藻则是大陆架、沿岸区(营养盐丰富水域)的重要种类。Stockner & Antia的初步研究表明<sup>[24]</sup>,在营养物含量过剩的水域中,超微藻类丰度降低。1990年Krupatkina也报道<sup>[25]</sup>,在贫营养海区,Pico浮游植物中的叶绿素含量占叶绿素总量的70%。李超伦、栾凤鹤对东海春季分级叶绿素a在不同海域的分布特点作了报道<sup>[13]</sup>,他们根据不同粒级的浮游植物所含叶绿素a对总叶绿素a的贡献大小,将调查海区划分为:A沿岸水控制的近岸内陆架区(50m等深线以内),Nano-是主要类群,其所含叶绿素a的浓度平均占总叶绿素a浓度的54%;B陆架变性水控制的内外陆架交接处的狭小区域,这一区域Net-最多,平均占总叶绿素a的55%;C黑潮水控制的外陆架区及外海,该区域中Pico-占绝对优势,其平均占总叶绿素a的70%。李超伦等认为水体中浮游植物的粒级结构受到营养盐、光照及其本身的沉降速度等多方面因素的影响,充足的营养盐对于Nano-和Net-的增长有促进作用,而对Pico-在浮游植物总生物量中所占的优势却有一定的控制压力。赵文等认为<sup>[19]</sup>,

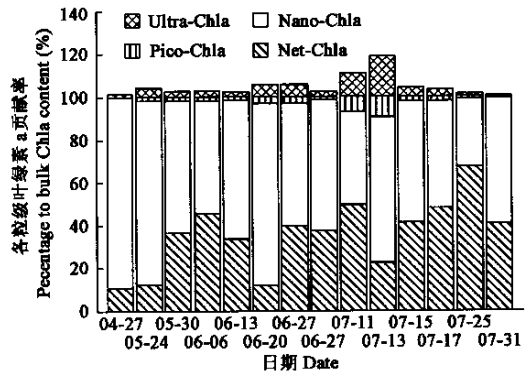


图2 各粒级叶绿素a对总叶绿素a贡献率的时间动态

Fig. 2 Temporal variations of percentage (%) of all size-fractionated chlorophyll-a contents to bulk chlorophyll-a contents



高度富营养化水体中超微藻对浮游植物现存量和生产量的贡献相对较低,浮游生物大小与水体营养状况和浮游生物食性鱼类的放养密度密切相关。

在本文研究中,超微藻对浮游植物现存量的贡献仅为1.3%,本文结果明显低于对贫营养水域的调查(表3)。对浮游植物而言,一般随着水体富营养化加剧,水中营养盐大量积累,使吸收速率增高、生长周期快的小型藻类占优势,但如果营养过剩,<2μm的超微藻类生物量就会减少。对本调查的富营养小水体来说,由于缺少捕食者,超微藻缺乏滤食和竞争压力,而水体营养物含量过剩,这些均是导致Pico-浮游植物现存量不高的原因所在。

3.2 环境因子对各粒径浮游植物的影响

通过对各粒径叶绿素含量以及贡献率与环境因子的相关分析表明,水体BOD<sub>5</sub>和TN/TP高,Net-chla所占的百分比则低,Nano-chla含量以及所占百分比高,反之,当水体BOD<sub>5</sub>、COD<sub>mn</sub>、TN/TP值减少后,Net-chla%增加,而Nano-chla含量以及Nano-chla%减少。水体中生化需氧量(BOD<sub>5</sub>)和化学需氧量(COD)均是用来反映需氧有机物的含量与水体污染的关系的指标,此两个指标越大,表明水体有机污染越严重。因此本相关分析结果说明Nano-和Net-chla%与水体的污染程度有密切关系,即水体的污染越严重,其Nano-chla含量及所占百分比会增多,而Net-粒级对chla贡献率则有降低;反之,若水体的污染程度减轻,Net-chla%则会增加,而Nano-chla含量以及Nano-chla%将减少(图3)。

营养盐是水体中藻类生长必不可少的因素,水中无机氮含量较大时,浮游植物对无机磷的摄取量亦大,并且这两种盐类含量以同样的方式变化,浮游植物的盛衰依赖于水域中营养盐的丰歉。从本研究的总N/总P比看,该均值为8.682,众多研究认为,藻类生长最适宜的氮磷质量比为7.2:1,通常将5~10氮磷质量比作为判别营养盐的粗略限制,可见本研究水体氮磷比是较适宜藻类生长的,实际上,本研究过程中浮游植物叶绿素a含量确实很高,最高时达到了350.12mg/m<sup>3</sup>。同时,随着氮磷比的降低或升高,net-贡献率会升高或下降,而nano-贡献率则正好与net-相反(图3)。

3.3 各粒径浮游植物对生态修复工程的响应

工程实施前后Net-chla含量的显著差异以及Net-chla%和Nano-chla%的非常显著差异表明,本研究水体Net-粒级和Nano-粒级Chla百分比值的变化并非是自然状态下的环境变化引起,而是由于恢复措施的实施所造成。不过,在进行理化各指标的分析中,所有测定项目在生态修复前后均无显著性差异(P>0.05),因此,Net-粒级和Nano-粒级Chla百分比值在修复前后的这种明显变化原因并非由所测的单项理化指标引起,可见,各粒径指标与环境因子的关系相当错综复杂,与生态系统的复杂性表现相同,因为生物与环境之间、生物与生物之间、理化因子之间都存在着变幻莫测的关联,如此复杂关系的产生正好恰如其分的表达了生态系统独特的瞬息万变之特征。

尽管net-和nano-粒级叶绿素a贡献率与COD<sub>mn</sub>、BOD<sub>5</sub>呈现非常显著的关联,但工程实施前后的COD<sub>mn</sub>和BOD<sub>5</sub>并无显著性差异,6月份两者都有异常的增加,观察其它指标在6月份的表现发现,6月13日温度由5月30日的21℃上升到27℃后,DO、pH都异常高,分别达到9mg/L和9.1,氮磷的含量6、7月份也比其它时间高,各项指标均差于其它时段,说明此时理化指标的异常与温度的大幅度上升有关,提示在温度的大幅度增加之时,应加大治理力度,以控制温度变化带来的水体质量恶化。

4 结语

表3 不同性质水域各粒级生物量的贡献比较

Table 3 Comparison with percentage (%) of all size-fractionated chlorophyll-a in different waters

水域性质 Waters character	Nano+Pico Chla%	Nano Chla%	Pico Chla%	参考文献 References
贫营养海洋 Oligotrophic ocean	—	—	70	[25]
中营养小湖泊 Meso-eutrophic	64.8	35.1	29.7	[19]
富营养小湖泊 Eutrophic little lake	70.2	67.1	3.1	[19]
富营养小河流 Eutrophic little river	61.9	47.3	14.6	[19]
本研究水体 This research water	67.3	65.2	2.1	本研究 This thesis

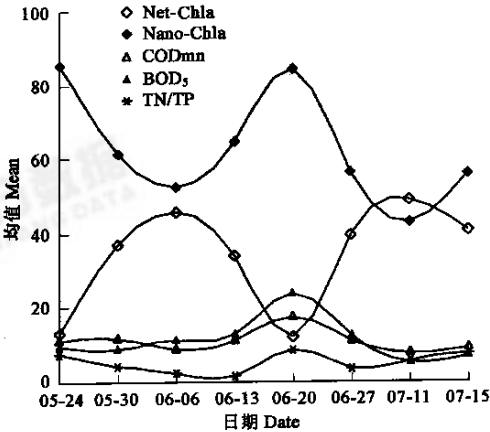


图3 各粒径叶绿素a贡献率与环境因子的关系

Fig. 3 Relationship between percentage (%) of all size-fractionated chlorophyll-a contents to bulk chlorophyll-a contents and the environmental factors

(1)本水体的浮游植物a的第一贡献者为Nano-粒级,Net-粒级也占有较高的比例,处于第2位,Pico-粒级所占份额最小。  
(2)Net-Chla%与BOD<sub>5</sub>和TN/TP呈非常显著负相关;Nano-Chla%与BOD<sub>5</sub>和TN/TP呈非常显著正相关;Net-Chla与

COD<sub>mn</sub>和TN/TP呈显著负相关。

(3)Net-chla 含量实施前与实施后有显著差异;Net-chla%和Nano-chla%实施前与实施后具有非常显著差异。

通过生物与生物之间,生物与环境之间的关系来评价生态系统的健康性已被越来越多的生态学者所采用。如果能对生态恢复进行中的水体建立快速评价恢复效果的指标,不仅可以为科学管理和正确选用恢复方案提供有力的证据,同时还可促进后续的管理决策进程。许多学者都在尝试建立一种生物方法以快速评价水体质量,但目前生物监测中,研究工作的重点依然放在分类和结构方面,由于此类方法比较烦琐,加上鉴定种名必须具相当专门的知识经验,故不易推广。叶绿素a的测定具有快速、简便等特点,利用叶绿素a对水体质量的监测主要见于对水体富营养化程度的评价中,尚未见利用粒径分级叶绿素a来监测水体质量的文献报道。作者认为,分粒径测定叶绿素a可以反映微型生物重要性的主要信息,尤其是微微型浮游生物,其生殖周期短,生长率快,在水体中的数量、生物量和生产力的大小可作为水生生态系统质量的一项重要指标<sup>[22,26,27]</sup>。分粒径叶绿素a百分率不仅能迅速反映环境变化,而且能对环境的改变作出明显的响应,加之分粒径测定简便快速,结果可靠,而且测定方法国内外较易趋于一致,研究结果具有非常高的可比性,因此,可以考虑利用各粒径分级叶绿素a百分比的变化来对水体质量或修复进程进行判断。当然,由于本研究的时间限制以及该生态修复的不彻底性,本文结果是否具有普遍性,能否用浮游植物粒径组成贡献率的变化来达到对水体演替方向的快速判断尚有待今后进一步的实验论证。

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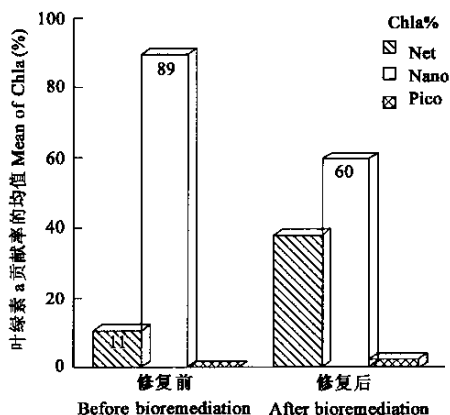


图4 各粒径级叶绿素a贡献率生态修复前后的变化

Fig. 4 Variations about percentage (%) of all size-fractionated chlorophyll-a contents to bulk chlorophyll-a contents and the environmental factors before and rear of the bioremediation  
前 before 修复前, before bioremediation; 后 after 修复后 after bioremediation

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